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(54) Title: DENDRITIC CELL MEMBRANE PROTEIN FIRE

(57) Abstract: The present invention relates to a protein (designated FIRE) which is preferentially expressed in dendritic cells stimulatory to T cells, macrophages and their precursors and to nucleic acid sequences encoding this protein. The invention also relates to uses of the protein and nucleic acids.

*Dendritic Cell Membrane Pr tein FIRE*

## FIELD OF THE INVENTION

The present invention relates to a protein (designated FIRE) which is preferentially expressed in dendritic cells stimulatory to T cells, macrophages and their precursors and to nucleic acid sequences encoding this protein. The invention also relates to uses of the protein and nucleic acids.

## BACKGROUND OF THE INVENTION

Dendritic cells (DC) are antigen presenting leukocytes which play a critical role in the initiation of immune responses. To stimulate naive T lymphocytes, which is an essential step in generating the immunological memory required for effective vaccination, it is crucial for antigen to be presented by DC. Over the last 8 years techniques have been developed to purify DC populations and lineages from mouse lymphoid organs. This DC purification protocol involves density centrifugation, depletion of contaminating cells with a monoclonal antibody cocktail and magnetic beads, and finally Fluorescent Activated Cell Sorting. Using an original version of this purification protocol, two DC populations in mouse spleen were identified, which are defined by their expression of two cell surface proteins: a lymphoid-lineage related $CD8^+Mac\text{-}1^-$ DC and a myeloid-lineage related $CD8^-Mac\text{-}1^+$ DC. These two populations differ in their interactions with T lymphocytes. Although the two DC populations displayed equivalent ability to stimulate T cells into cell cycle, they differed in their ability to induce the production of cytokines such as IL-2 and IL-3, which are critical for the induction of an effective immune response (1-5). The myeloid related $CD8^-Mac\text{-}1^+$DC are much more efficient in cytokine induction than the lymphoid related $CD8^+Mac\text{-}1^-$ DC. Other differences between the DC are the levels of cytokines they themselves produce (such as IL-12) which, potentially, could regulate the nature as well as the quality of cytokines they induce in the activated T cells. The molecular mechanisms which underpin these differences in interactions between the two DC populations and T lymphocytes are unknown. Presumably myeloid DC differentially express molecules which enable them to stimulate T lymphocytes to produce certain cytokines more efficiently than do lymphoid DC, or alternatively lymphoid

DC differentially express molecules which inhibit the stimulation of T lymphocytes. To address this question the present inventors compared gene expression in the two DC populations using the technique of Representational Difference Analyses (RDA)(6).

Briefly, RDA identifies differential gene expression between two given cell types by using successive rounds of a combination of PCR and subtractive hybridisation which generates DNA fragments of putatively differentially expressed genes (6).

A full length clone encoding a novel mouse gene was obtained using conventional molecular biological techniques, involving the RDA generated fragments. This sequence was designated "FIRE" and encodes a 681 amino acid protein. Analyses of the FIRE sequence shows that it is a novel member of the recently described EGF/TM7 superfamily (7). The mouse sequence was then used to isolate the equivalent human FIRE sequence.

## SUMMARY OF THE INVENTION

Accordingly, in a first aspect the present invention consists in an isolated polypeptide, the polypeptide comprising:-

(i) an amino acid sequence as set out in SEQ ID NO:1, or

(ii) an amino acid sequence having at least 50% identity to the amino acid sequence set out in SEQ ID NO:1, or

(iii) a functional fragment of (i) or (ii).

In a preferred embodiment of the first aspect, the polypeptide has a sequence of at least 70%, more preferably at least 80% and most preferably at least 90% identity with the sequence shown in SEQ ID NO:1.

In a second aspect, the present isolated polypeptide, the polypeptide comprising:-

(i) an amino acid sequence as set out in SEQ ID NO:2, or

(ii) an amino acid sequence having at least 50% identity to the amino acid sequence set out in SEQ ID NO:2, or

(iii) a functional fragment of (i) or (ii).

In a preferred embodiment of the second aspect, the polypeptide has a sequence of at least 70%, more preferably at least 80% and most preferably at least 90% identity with the sequence shown in SEQ ID NO:2.

In a further preferred embodiment of the first and second aspects, the polypeptide is expressed on dendritic cells.

The term "functional fragment" as used herein is intended to cover fragments of the polypeptide which retain at least 10% of the biological activity of the complete polypeptide. In particular this term is used to encompass fragments which show immunological cross-reactivity with the entire polypeptide, eg ligands which interact with the fragment also interact with the complete polypeptide.

In a third aspect the present invention consists in an isolated ligand, the ligand being directed against the polypeptide of the first aspect of the present invention.

The ligand may be an inorganic or organic molecule. In one preferred embodiment the ligand is an antibody or the binding portion thereof.

In a fourth aspect the present invention provides an isolated nucleic acid molecule, the nucleic acid molecule encoding a polypeptide of the first or second aspects.

In a fifth aspect, the present invention provides an isolated nucleic acid molecule, the nucleic acid molecule comprising:-

(i) a sequence as set out in SEQ ID NO:3, or

(ii) a sequence having at least 60% identity to the sequence set out in SEQ ID NO:3, or

(iii) a sequence which hybridises to the sequence set out in SEQ ID NO:3 under stringent conditions.

In a preferred embodiment of the fifth aspect, the nucleic acid molecule comprises a sequence of at least 70%, more preferably at least 80% and most preferably at least 90% identity with the sequence shown in SEQ ID NO:3.

In a further preferred embodiment of the fifth aspect, the isolated nucleic acid molecule has at least 95% identity to the nucleotide sequence shown in SEQ ID NO:3, preferably within the region from nucleotide 218 to 2260.

In a sixth aspect the present invention provides an isolated nucleic acid molecule, the nucleic acid molecule comprising:-

(i) a sequence as set out in SEQ ID NO:4, or

(ii) a sequence having at least 60% identity to the sequence set out in SEQ ID NO:4, or

(iii) a sequence which hybridises to the sequence set out in SEQ ID NO:4 under stringent conditions.

In a preferred embodiment of the sixth aspect, the nucleic acid molecule comprises a sequence of at least 70%, more preferably at least 80% and most preferably at least 90% identity with the sequence shown in SEQ ID NO:4.

In a further preferred embodiment of the sixth aspect, the isolated nucleic acid molecule has at least 95% identity to the nucleotide sequence shown in SEQ ID NO:4 within the region from nucleotide 1 to 1903.

In a seventh aspect the present invention provides an isolated nucleic acid molecule, the nucleic acid molecule encoding the binding region of a ligand of the third aspect.

In a further preferred embodiment of the present invention, the nucleic acid molecules of the present invention are preferably less than 5000 nucleotides, however, they may be less than 1000 or 500 nucleotides in length. Preferably, the nucleic acid molecules of the present invention are at least 18 nucleotides in length.

An "isolated" polypeptide or ligand refers to a polypeptide or ligand that has been substantially isolated from other proteins, lipids, nucleic acids and other contaminants.

An "isolated" nucleic acid molecule refers to a nucleic acid molecule that is one or both of the following: not immediately contiguous with both of the coding sequences with which it is immediately contiguous in the naturally occurring genome of the organism from which the nucleic acid is derived; or which is substantially free of other nucleic acid, proteins, lipids, and other contaminants.

In an eighth aspect the present invention consists in a composition for use in raising or lowering an immune response in a subject, the composition comprising a ligand of the third aspect of the present invention and an antigen and optionally a carrier and/or adjuvant.

In a preferred embodiment the antigen is associated with the ligand. The antigen may be associated with the ligand by any suitable means known in the art. Suitable methods for associating the ligand and antigen are described, for example, in Cox, J. and Coulter, A.R. (1999) Biodrugs 12:439-453.

In a further preferred embodiment, the antigen is conjugated to the ligand.

It will be appreciated by those skilled in the art that in the context of the eighth aspect, any antigen of interest may be used in the composition. For example, the antigen may be derived from an infectious pathogen or from a tumour cell.

In a ninth aspect the present invention consists in a composition for use in raising or lowering an immune response in a subject, the composition comprising a nucleic acid molecule and a carrier, the nucleic acid molecule comprising a first sequence encoding a ligand of the third aspect of the present invention and a second sequence encoding an antigen.

In a tenth aspect the present invention consists in a method of screening compounds for immunological regulatory activity, the method comprising reacting the compound with the polypeptide or peptide of the first aspect of the invention and measuring interaction between the compound and the polypeptide or peptide.

As will be appreciated by those skilled in the art, the polypeptides, peptides and nucleic acid molecules of the present invention provide useful markers of subgroups of dendritic cells and antigen presenting cells (such as macrophages). They also provide useful markers of dendritic cell precursors.

The nucleic acid molecules of the present invention may also be used as tools to analyse the properties and functions of the FIRE gene/protein. For example, the nucleic acid molecules may be used to generate animal models, preferably mouse models, wherein the animals lack functional FIRE genes. Alternatively, the nucleic acid molecules may be introduced and expressed in cells in which the FIRE gene is not normally expressed.

It will also be appreciated that the nucleic acid molecules of the present invention may be used to isolate regulatory regions (such as the promoter region) of the FIRE gene. Such regulatory regions may be used to selectively express exogenous genes in dendritic or antigen presenting cells.

The ligands of the present invention may be used to isolate dendritic cells, dendritic cell precursors, or other antigen presenting cells, from biological samples (eg. from blood). Accordingly, these ligands may be used in various immunisation processes. For example, the cells which are isolated from a patient through use of these ligands may be grown *in vitro*, exposed to one or more antigens, and then introduced back into the patient.

The ligands of the present invention may also be used to modulate immune responses by interfering with the function, migration or maturation

of dendritic or antigen presenting cells. Ligands which act as agonists or antagonists may be useful in the modulation of immune responses. For example, ligands of the present invention may be administered to patients under conditions such that the ligands bind to and interfere with the function of myeloid dendritic cells, with the result that antigen processing is undertaken by lymphoid dendritic cells. This may lead to immune suppression and anergy, a desirable outcome in the treatment of allergies and autoimmune disorders.

The ligands of the present invention may also be used to target molecules, such as vaccine components, to dendritic or antigen presenting cells. Suitable methods for targeting will be known to those skilled in the art. Non-limiting examples of suitable taragetting methods are described in WO 98/44129.

Throughout this specification the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

## BRIEF DESCRIPTION OF THE FIGURES

Figure 1: FIRE-FLAG is presented on the surface of stably transfected CHO cells as detected by immunofluorescent staining and flow cytometry using anti-FLAG mAb (IC7) and anti-mouse-PE. The filled histograph represents CHO cells transfected with the neomycin resistance gene only, stained with anti-FLAG mAb whilst the hollow histograph are CHO-FIRE-FLAG cells staining positive for the FLAG epitope.

Figure 2 shows the full length cDNA sequence of mouse FIRE and the translated protein sequence.

Figure 3 shows a comparison of the protein sequence of FIRE with members of the EGF/TM7 superfamily including human Emr1 (SEQ ID NO:5); mouse EMR1 (SEQ ID NO:6) and human CD97 (SEQ ID NO:7).

Figure 4: Immunoflurescent staining of FIRE on spenic DC, splenic macrophages and blood mononuclear cells. A) DC were extracted and purified from spleens of C57BL/6 mice and stained with anti-CD11c, anti-CD4, anti-CD8 and anti-FIRE. The cells that were gated on expressed high levels of CD11c and high forward scatter. Dead cells staining with PI were gated out using the FL5 channel. DC that failed to express CD4 and CD8 expressed the highest level of FIRE. CD4+ and CD8+ DC express lower levels of FIRE. Smooth line denotes background control staining and dotted line indicates staining with FIRE. B) Macrophages were extracted and purified from spleens of C57BL/6 mice and stained with anti-CD11b, anti-F4/80, and anti-FIRE. C) Macrophages, defined as the population of cells that express high levels of both F4/80 and CD11b, also express FIRE. Smooth line denotes background control staining and dotted line indicates staining with FIRE. D) Blood mononuclear cells were obtained from C57BL/6 mice by centrifugation over a gradient (lympholyte M), then depleted of cells expressing CD3, GR-1, TER119, Thy1.1 and B220. A large proportion of the remaining blood mononuclear cells expressed FIRE.

Figure 5: *In vitro* culture of FIRE+ blood mononuclear cells gives rise to a high proportion of CD11c positive cells. Peripheral blood mononuclear cells were isolated as described above. Cells were stained with anti-FIRE mAb then sorted on the basis of FIRE expression. Both FIRE+ (A and B) and FIRE- (C and D) cells were incubated in medium alone (A and C) or medium plus IL-4, FL3L, TNF-α, and GM-CSF (B and D).

Figure 6 shows the cDNA sequence of human FIRE and the translated protein sequence.

## DETAILED DESCRIPTION OF THE INVENTION

In order that the nature of the present invention may be more clearly understood preferred forms thereof will now be described with reference to the following non-limiting Examples.

### General Molecular Biology

Unless otherwise indicated, the recombinant DNA techniques utilized in the present invention are standard procedures, well known to those skilled in the art. Such techniques are described and explained throughout the literature in sources such as, J. Perbal, A Practical Guide to Molecular Cloning, John Wiley and Sons (1984), J. Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbour Laboratory Press (1989), T.A. Brown (editor), Essential Molecular Biology: A Practical Approach, Volumes 1 and 2, IRL Press (1991), D.M. Glover and B.D. Hames (editors), DNA Cloning: A Practical Approach, Volumes 1-4, IRL Press (1995 and 1996), and F.M. Ausubel et al. (Editors), Current Protocols in Molecular Biology, Greene Pub. Associates and Wiley-Interscience (1988, including all updates until present) and are incorporated herein by reference.

### Protein Variants

Amino acid sequence variants can be prepared by introducing appropriate nucleotide changes into DNA, or by *in vitro* synthesis of the desired polypeptide. Such variants include, for example, deletions, insertions or substitutions of residues within the amino acid sequence. A combination of deletion, insertion and substitution can be made to arrive at the final construct, provided that the final protein product possesses the desired characteristics. The amino acid changes also may alter post-translational processes such as changing the number or position of glycosylation sites, altering the membrane anchoring characteristics, altering the intra-cellular location by inserting, deleting or otherwise affecting the transmembrane sequences of the native protein, or modifying its susceptibility to proteolytic cleavage.

In designing amino acid sequence variants, the location of the mutation site and the nature of the mutation will depend on characteristic(s) to be modified. The sites for mutation can be modified individually or in series, e.g., by (1) substituting first with conservative amino acid choices and

then with more radical selections depending upon the results achieved, (2) deleting the target residue, or (3) inserting residues of other ligands adjacent to the located site.

A useful method for identification of residues or regions for mutagenesis is called "alanine scanning mutagenesis" as described by Cunningham and Wells (*Science* (1989) 244: 1081-1085). Here, a residue or group of target residues are identified (e.g., charged residues such as Arg. Asp, His, Lys, and Glu) and replaced by a neutral or negatively charged amino acid (most preferably alanine or polyalanine) to affect the interaction of the amino acids with the surrounding aqueous environment in or outside the cell. Those domains demonstrating functional sensitivity to the substitutions then are refined by introducing further or other variants. Thus, while the site for introducing an amino acid sequence variation is predetermined, the nature of the mutation *per se* need not be predetermined. For example, to optimise the performance of a mutation at a given site, alanine scanning or random mutagenesis may be conducted at the target codon or region and the expressed variants are screened for the optimal combination of desired activity.

There are two principal variables in the construction of amino acid sequence variants; the location of the mutation site and the nature of the mutation. These may represent naturally occurring alleles or predetermined mutant forms made by mutating the DNA either to arrive at an allele or a variant not found in nature. In general, the location and nature of the mutation chosen will depend upon the characteristic to be modified.

Amino acid sequence deletions generally range from about 1 to 30 residues, more preferably about 1 to 10 residues and typically about 1 to 5 contiguous residues.

Amino acid sequence insertions include amino and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Other insertional variants include the fusion of the N- or C-terminus of the proteins to an immunogenic polypeptide e.g. bacterial polypeptides such as betalactamase or an enzyme encoded by the *E. coli trp* locus, or yeast protein, bovine serum albumin, and chemotactic polypeptides. C-terminal fusions with proteins having a

long half-life such as immunoglobulin constant regions (or other immunoglobulin regions), albumin, or ferritin, are included.

Another group of variants are amino acid substitution variants. These variants have at least one amino acid residue in the protein molecule removed and a different residue inserted in its place. The sites of greatest interest for substitutional mutagenesis include sites identified as the active site(s). Other sites of interest are those in which particular residues obtained from various species are identical. These positions may be important for biological activity. These sites, especially those falling within a sequence of at least three other identically conserved sites, are substituted in a relatively conservative manner. Such conservative substitutions are shown in Table 1 under the heading of "preferred substitutions". If such substitutions result in a change in biological activity, then more substantial changes, denominated exemplary substitutions in Table 1, or as further described below in reference to amino acid classes, are introduced and the products screened.

TABLE 1

| **Original Residue** | **Exemplary Substitutions** | **Preferred Substitutions** |
|---|---|---|
| Ala (A) | val; leu; ile | val |
| Arg (R) | lys; gln; asn | lys |
| Asn (N) | gln; his; lys; arg | gln |
| Asp (D) | glu | glu |
| Cys (C) | ser | ser |
| Gln (Q) | asn | asn |
| Glu (E) | asp | asp |
| Gly (G) | pro | pro |
| His (H) | asn; gln; lys; arg | arg |
| Ile (I) | leu; val; met; ala; phe norleucine | leu |
| Leu (L) | norleucine, ile; val; met; ala; phe | ile |

| | | |
|---|---|---|
| Lys (K) | arg; gln; asn | arg |
| Met (M) | leu; phe; ile; | leu |
| Phe (F) | leu; val; ile; ala | leu |
| Pro (P) | gly | gly |
| Ser (S) | thr | thr |
| Thr (T | ser | ser |
| Trp (W) | tyr | tyr |
| Tyr (Y) | trp; phe; thr; ser | phe |
| Val (V) | ile; leu; met; phe; ala; norleucine | leu |

**Mutants, Variants and Homology - Proteins**

Mutant polypeptides will possess one or more mutations which are deletions, insertions, or substitutions of amino acid residues. Mutants can be either naturally occurring (that is to say, purified or isolated from a natural source) or synthetic (for example, by performing site-directed mutagensis on the encoding DNA). It is thus apparent that polypeptides of the invention can be either naturally occurring or recombinant (that is to say prepared using recombinant DNA techniques).

An allelic variant will be a variant that is naturally occurring within an individual organism.

Protein sequences are homologous if they are related by divergence from a common ancestor. Consequently, a species homologue of the protein will be the equivalent protein which occurs naturally in another species. Within any one species a homologue may exist as numerous allelic variants, and these will be considered homologues of the protein. Allelic variants and species homologues can be obtained by following standard techniques known to those skilled in the art. Preferred species homologues include those obtained from representatives of the same Phylum, more preferably the same Class and even more preferably the same Order.

A protein at least 50% identical, as determined by methods well known to those skilled in the art (for example, the method described by Smith, T.F. and Waterman, M.S. (1981) Ad. Appl Math., 2: 482-489, or Needleman, S.B. and Wunsch, C.D. (1970) J. Mol. Biol., 48: 443-453), to that of the present invention are included in the invention, as are proteins at least 70% or 80%

and more preferably at least 90% identical to the protein of the present invention. This will generally be over a region of at least 20, preferably at least 30, contiguous amino acids.

**Mutants, Variants and Homology - Nucleic Acids**

Mutant polynucleotides will possess one or more mutations which are deletions, insertions, or substitutions of nucleotide residues. Mutants can be either naturally occurring (that is to say, isolated from a natural source) or synthetic (for example, by performing site-directed mutagensis on the DNA). It is thus apparent that polynucleotides of the invention can be either naturally occurring or recombinant (that is to say prepared using recombinant DNA techniques).

An allelic variant will be a variant that is naturally occurring within an individual organism.

Nucleotide sequences are homologous if they are related by divergence from a common ancestor. Consequently, a species homologue of the polynucleotide will be the equivalent polynucleotide which occurs naturally in another species. Within any one species a homologue may exist as numerous allelic variants, and these will be considered homologues of the polynucleotide. Allelic variants and species homologues can be obtained by following standard techniques known to those skilled in the art. Preferred species homologues include those obtained from representatives of the same Phylum, more preferably the same Class and even more preferably the same Order.

A polynucleotide at least 60% identical, as determined by methods well known to those skilled in the art (for example, the method described by Smith, T.F. and Waterman, M.S. (1981) Ad. Appl Math., 2: 482-489, or Needleman, S.B. and Wunsch, C.D. (1970) J. Mol. Biol., 48: 443-453), to that of the present invention are included in the invention, as are proteins at least 80% or 90% and more preferably at least 95% identical to the polynucleotide of the present invention. This will generally be over a region of at least 60, preferably at least 90, contiguous nucleotide residues.

**Antibody Production**

Antibodies, either polyclonal or monoclonal, which are specific for a protein of the present invention can be produced by a person skilled in the

art using standard techniques such as, but not limited to, those described by Harlow et al. Antibodies: A Laboratory Manual, Cold Springs Harbor Laboratory Press (1988), and D. Catty (editor), Antibodies: A Practical Approach, IRL Press (1988).

Various procedures known in the art may be used for the production of polyclonal antibodies to epitopes of a protein. For the production of polyclonal antibodies, a number of host animals are acceptable for the generation of antibodies by immunization with one or more injections of a polypeptide preparation, including but not limited to rabbits, mice, rats, etc. Various adjuvants may be used to increase the immunological response in the host animal, depending on the host species, including but not limited to Freund's (complete and incomplete), mineral gels such as aluminium hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, oil emulsions, keyhole lympet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and corynebacterium parvum.

A monoclonal antibody to an epitope of a protein may be prepared by using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include but are not limited to the hybridoma technique originally described by Kohler and Milstein (1975, Nature 256, 493-497), and the more recent human B-cell hybridoma technique (Kesber *et al.* 1983, Immunology Today 4:72) and EBV-hybridoma technique (Cole *et al.* 1985, Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc. pp. 77-96). In addition, techniques developed for the production of "chimeric antibodies" by splicing the genes from an antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity may be used (Morrison *et al.* 1984, Proc. Natl. Acad. Sci., 81:6851-6855; Neuberger *et al.* 1984 Nature 312:604-608; Takeda *et al.* 1985 Nature 31:452-454). Alternatively, techniques described for the production of single chain antibodies (U.S. Patent 4,946,778) can be adapted to produce 4-specific single chain antibodies.

Recombinant human or humanized versions of monoclonal antibodies are a preferred embodiment for human therapeutic applications. Humanized antibodies may be prepared according to procedures in the literature (e.g. Jones *et al.* 1986, Nature 321:522-25; Reichman *et al.* 1988, Nature

332:323-27; Verhoeyen *et al.* 1988, Science 239:1534-36). The recently described "gene conversion mutagenesis" strategy for the production of humanized monoclonal antibody may also be employed in the production of humanized antibodies (Carter *et al.* 1992 Proc. Natl. Acad. Sci. U.S.A. 89:4285-89). Alternatively, techniques for generating the recombinant phage library of random combinations of heavy and light regions may be used to prepare recombinant antibodies (e.g. Huse *et al.* 1989 Science 246:1275-81).

Antibody fragments which contain the idiotype of the molecule such as Fv $F(ab^1)$ and $F(ab^2)$ may be generated by known techniques. For example, such fragments include but are not limited to: the F(ab) E2 fragment which can be produced by pepsin digestion of the intact antibody molecule; the Fab' fragments which can be generated by reducing the disulfide bridges of the F(ab')2 fragment, and the two Fab fragments which can be generated by treating the antibody molecule with papain and a reducing agent. Alternatively, Fab expression libraries may be constructed (Huse *et al.* 1989, Science 246:1275-1281) to allow rapid and easy cloning of a monoclonal Fab fragment with the desired specificity to a protein.

**Adjuvants and Carriers**

Pharmaceutically acceptable carriers or diluents include those used in compositions suitable for oral, rectal, nasal, topical (including buccal and sublingual), vaginal, parenteral (including subcutaneous, intramuscular, intravenous, intradermal, intrathecal and epidural) administration. They are non-toxic to recipients at the dosages and concentrations employed. Representative examples of pharmaceutically acceptable carriers or diluents include, but are not limited to water, isotonic solutions which are preferably buffered at a physiological pH (such as phosphate-buffered saline or Tris-buffered saline) and can also contain one or more of, mannitol, lactose, trehalose, dextrose, glycerol, ethanol or polypeptides (such as human serum albumin). The compositions may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy.

As mentioned above the composition may include an adjuvant. As will be understood an "adjuvant" means a composition comprised of one or more substances that enhances the immunogenicity and efficacy of a vaccine composition. Non-limiting examples of suitable adjuvants include squalane

and squalene (or other oils of animal origin); block copolymers; detergents such as Tween®-80; Quil® A, mineral oils such as Drakeol or Marcol, vegetable oils such as peanut oil; *Corynebacterium*-derived adjuvants such as *Corynebacterium parvum*; *Propionibacterium*-derived adjuvants such as *Propionibacterium acne*; *Mycobacterium bovis* (Bacille Calmette and Guerin or BCG); interleukins such as interleukin 2 and interleukin 12; monokines such as interleukin 1; tumour necrosis factor; interferons such as gamma interferon; combinations such as saponin-aluminium hydroxide or Quil-A aluminium hydroxide; liposomes; ISCOM adjuvant; mycobacterial cell wall extract; synthetic glycopeptides such as murarmyl dipeptides or other derivatives; Avridine; Lipid A derivatives; dextran sulfate; DEAE-Dextran or with aluminium phosphate; carboxypolymethylene such as Carbopol' EMA; acrylic copolymer emulsions such as Neocryl A640 (e.g. U.S. Pat. No. 5,047,238); vaccinia or animal poxvirus proteins; sub-viral particle adjuvants such as cholera toxin, or mixtures thereof.

**Gene/DNA Isolation**

The DNA encoding a protein may be obtained from any cDNA library prepared from tissue believed to express the gene mRNA and to express it at a detectable level. DNA can also be obtained from a genomic library.

Libraries are screened with probes or analytical tools designed to identify the gene of interest or the protein encoded by it. For cDNA expression libraries, suitable probes include monoclonal or polyclonal antibodies that recognize and specifically bind the protein; oligonucleotides of about 20-80 bases in length that encode known or suspected portions of cDNA from the same or different species; and/or complementary or homologous cDNAs or fragments thereof that encode the same or a hybridizing gene. Appropriate probes for screening genomic DNA libraries include, but are not limited to, oligonucleotides; cDNAs or fragments thereof that encode the same or hybridizing DNA including expressed sequence tags and the like; and/or homologous genomic DNAs or fragments thereof. Screening the cDNA or genomic library with the selected probe may be conducted using standard procedures as described in chapters 10-12 of Sambrook *et al.*

An alternative means to isolate a gene encoding the protein of interest is to use polymerase chain reaction (PCR) methodology as described in

section 14 of Sambrook *et al.* This method requires the use of oligonucleotide probes that will hybridize to the gene.

The oligonucleotide sequences selected as probes should be of sufficient length and sufficiently unambiguous that false positives are minimized. The actual nucleotide sequence(s) is usually based on conserved or highly homologous nucleotide sequences or regions of the gene. The oligonucleotides may be degenerate at one or more positions. The use of degenerate oligonucleotides may be of particular importance where a library is screened from a species in which preferential codon usage in that species is known. The oligonucleotide must be labelled such that it can be detected upon hybridization to DNA in the library being screened. The preferred method of labelling is to use ($\alpha$-$^{32}$P)- dATP with polynucleotide kinase, as is well known in the art, to radiolabel the oligonucleotide. However, other methods may be used to label the oligonucleotide, including, but not limited to, biotinylation or enzyme labelling.

DNA encompassing all the protein coding sequence is obtained by screening selected cDNA or genomic libraries, and if necessary, using conventional primer extension procedures as described in section 7.79 of Sambrook *et al.*, to detect precursors and processing intermediates of mRNA that may not have been reverse-transcribed into cDNA.

Another alternative method for obtaining the gene of interest is to chemically synthesize it using one of the methods described in Fingels *et al.* (*Agnew Chem. Int. Ed. Engl. 28:* 716-734, 1989). These methods include triester, phosphite, phosphoramidite and H-Phosphonate methods, PCR and other autoprimer methods, and oligonucleotide syntheses on solid supports. These methods may be used if the entire nucleic acid sequence of the gene is known, or the sequence of the nucleic acid complementary to the coding strand is available, or alternatively, if the target amino acid sequence is known, one may infer potential nucleic acid sequences using known and preferred coding residues for each amino acid residue.

**Nucleic acid hybridisation**

The polynucleotide sequence of the present invention may hybridise to the sequence set out in SEQ ID NO:3 or SEQ NO:4 under high stringency. As used herein, stringent conditions are those that (i) employ low ionic strength and high temperature for washing after hybridization, for example, 0.1 x SSC

and 0.1% (w/v) SDS at 50°C; (ii) employ during hybridization conditions such that the hybridization temperature is 25°C lower than the duplex melting temperature of the hybridizing polynucleotides, for example 1.5 x SSPE, 10% (w/v) polyethylene glycol 6000, 7% (w/v) SDS, 0.25 mg/ml fragmented herring sperm DNA at 65°C; or (iii) for example, 0.5M sodium phosphate, pH 7.2, 5mM EDTA, 7% (w/v) SDS and 0.5% (w/v) BLOTTO at 70°C; or (iv) employ during hybridization a denaturing agent such as formamide, for example, 50% (v/v) formamide with 5 x SSC, 50mM sodium phosphate (pH 6.5) and 5 x Denhardt's solution at 42°C; or (v) employ, for example, 50% (v/v) formamide, 5 x SSC, 50mM sodium phosphate (pH 6.8), 0.1% (w/v) sodium pyrophosphate, 5 x Denhardt's solution, sonicated salmon sperm DNA (50$\mu$g/ml) and 10% dextran sulphate at 42°C.

## EXPERIMENTAL DETAILS

## MATERIALS AND METHODS

### 1. Isolation of Dendritic cells (DC)

The procedure for the isolation of DC subpopulations has been described elsewhere in detail (Vremec *et al*, (1992). J. Exp. Med. 176: 47-58; Kronin *et al*. (1996). J. Immunol, 157; 3819). Briefly, spleens were digested with collagenase (1mg/ml; Worthington type II) and DNAase at room temperature for 20 min, followed by EDTA treatment for 5 min to disrupt DC-T cell complexes. Remaining procedures were conducted at 4°C. Low density cells were enriched by centrifugation for 10 min in Nycodenz medium (1.077 g/cm$^3$ mouse osmolarity). The low density cells were incubated with a mixture of mAb consisting of: anti-CD3, KT3-1.1; anti-CD4, GK1.5; anti-Th1.2, 30-H12; anti-Gr-1, RB68C5; anti-F4/80, anti-B220, RA36B2; and anti-erythrocytes, TER119. All the mAb were used at pre-titrated levels. Antibody coated cells were depleted with anti-rat IgG-conjugated magnetic beads, used at 5:1 bead-to-cell-ratio. The remaining cells were stained with fluorochrome-conjugated anti-CD11c and anti-CD8$\alpha$ mAb and propidium iodide (to label and exclude dead cells). Populations of > 95% pure viable CD11c$^+$ CD8$\alpha^+$ and CD11c$^+$ CD8$\alpha^-$ DC were isolated by sorting on MoFlow (Cytomation Inc.). Cells were snap frozen and stored at -70°C until used to extract RNA.

**2. RDA (Representational Difference Analysis)**

RNA was extracted using QuickPrep Micro mRNA Purification Kit (Pharmacia Biotech) and cDNA was synthesised (cDNA Synthesis Kit, Boehringer Mannheim Biochemica) according to the manufacturer's instruction. The cDNA RDA method was essentially as described by Hubank and Schatz (Nuc. Acid. Res. 22: 5640-5648, 1994). Minor alternations to this protocol include the amount of starting RNA. Due to the scarcity of the two DC populations, a total of 5 x $10^5$ $CD8^-Mac\text{-}1^+$ DC and 1.8 x $10^6$ $CD8^+Mac\text{-}1^-$ DC were used to extract mRNA. The synthesised double stranded cDNA was then digested with *Dpn*II and purified by phenol extraction and ethanol precipitation in the presence of 2 µg glycogen. Digested cDNA was annealed with R-Bgl-24 and R-Bgl-12 and ligated with T4 DNA Ligase (1200 units) at 14°C for 12-16 h. To compensate for the fact that three-fold more $CD8^+Mac\text{-}1^-$ DC were used to obtain mRNA, the ligated cDNA was diluted by a factor of three. Aliquots (1 µl) of the ligation mixture were amplified in multiple 100 µl polymerase chain reactions (PCR) using R-Bgl-24. The PCR reaction contained; 66mM Tris-HCl (pH8.8), 4mM $MgCl_2$, 16mM $(NH_4)_2SO_4$; 33 µg/ml BSA, dATP, dCTP, dGTP, and dTTP (all 0.3 mM) and 2 µg R-Bgl-24 primer. The R-Bgl-12 oligonucleotide was melted away at 72°C (3 min) and the 3'ends were filled in with 5 U Taq DNA polymerase (Perkin Elmer) at 72°C (5 min). Twenty cycles of amplification were performed (1min, 95°C; 3 min, 72°C). Amplification products were visualised on a 1.3% agarose gel containing ethidium bromide which confirmed that each sample gave rise to a similar concentration of representations. Products of each representation were then combined, phenol extracted, ethanol precipitated and resuspended in TE at 0.5 µg/ml. The R-adapters were removed from the representation with *Dpn*II and the digest was phenol extracted and ethanol precipitated to form the driver. Twenty micrograms of this driver was further gel-purified on a 1.2% TAE agarose gel, and the product, which was now free of the R-adapter, was isolated using QIAEX (Qiagen). This formed the "tester" of which 2 µg were ligated to the J-Bgl-12/24 adapter in the same manner as described above. For the first subtractive hybridisation step, 0.4 µg J-ligated tester ($CD8^+$ DC) was mixed with 40 µg of driver ($CD8^-$ DC) and *visa versa*. The mixture was phenol extracted, ethanol precipitated, and resuspended in 4 µl of EEx3 buffer (30 mM EPPS (Sigma), pH 8.0; 3 mM EDTA). The solution was

overlayed with mineral oil and the DNA was denatured for 6 min (98°C). The salt concentration was adjusted with 1 µl of 5M NaCl and the sample was allowed to anneal for 20 h (67°C). The hybridised sample was diluted with 8 µl TE (10mM Tris, 1mM EDTA, pH8.0) containing 5 mg/ml yeast RNA and then resuspended in a total volume of 400 µl TE. For each subtraction, four 200 µl PCR reactions containing 20 µl of hybridisation mix were set up as previously, but the primer was omitted. Again, the 12-mer oligo (R-Bgl-12) was melted away and 3' ends were filled using Taq DNA polymerase, then 2 µg of J-24-mer was added. After ten cycles of amplification, the four reactions were pooled, phenol extracted, isopropanol precipitated, and resuspended in 40 µl of 0.2xTE. Twenty microlitres of the product was digested with 20 U of mung bean nuclease and the reaction was stopped after 30 min by the addition of 50 mM Tris-HCl (pH8.9). The digest was heated to 98°C (5 min), chilled on ice then used in the final amplification. Four PCRs were conducted per hybridisation. Each PCR containing 20 µl of MBN-treated product and 2µg J-Bgl-24 was heated to 80°C, before 5 U of Taq DNA polymerase was added and further 18 amplification cycles were performed. The four reactions were pooled, phenol extracted, isopropanol precipitated, and resuspended at 0.5 µg/µl, giving the first differential product (DP1). The J-adapters were changed with N-Bgl-12/24 adapter and the process was repeated, with the exception that 50 ng tester was mixed with 40 µg of driver (i.e. 1:800). To generate the final DP3 product, 100 pg of J-ligated DP2 was mixed with 40 µg driver (i.e. 1:400,000) and the process was repeated except that the final amplification was performed for 22 cycles (70°C, 3 min; 95°C. 1 min).

**3. Protein expression of FIRE domains**

**3.1. Oligonucleotides**

Four cDNA constructs consisting of various extracellular domains of the FIRE clones were amplified by PCR using the following strategy:

(i) FIRE EGF domain 1;

Forward primer (5'-3'): CTAC GGATCC AAT ATT TCA GCT TCC TGT CC (SEQ ID NO:8);

Reverse primer (5'-3'): CGCG AAGCTT TCA ATC TTG ACA TTT CTC ATG G (SEQ ID NO:9).

(ii) FIRE EGF domain 2 Forward primer(5'-3'): GACG GGATCC AAT GAG TGT CTA CTG AAA GAA TTG (SEQ ID NO:10);
Reverse primer (5'-3'): ACCG AAGCTT TCA GCT CTT GTT CAC ATA ACA ATC (SEQ ID NO:11).

(iii) FIRE EGF domain 1 & 2;
Forward primer (5'-3'): CTAC GGATCC AAT ATT TCA GCT TCC TGT CC (SEQ ID NO:12);
Reverse primer (5'-3'): ACCG AAGCTT TCA GCT CTT GTT CAC ATA ACA ATC (SEQ ID NO:13).

(iv) FIRE Hinge;
Forward primer (5'-3'): ACAC GGATCC ACT TTG GGA GTA CTG AGT GAA (SEQ ID NO:14);
Reverse primer (5'-3'): CGCT AAGCTT TCA TAG AGC CAT GAG CAC AGC A (SEQ ID NO:15).

**3.2. PCR Protocol**

The oligonucleotide pairs listed above were used to amplify the corresponding FIRE domains from BlueScript plasmid DNA (1 µl of 1:10 dilution) containing FIRE cDNA inserts.

The final concentration of each oligonucleotide in the PCR was 400 nM, magnesium concentration was 2 mM and Elongase (Gibco-BRL) was used as the polymerase in all reactions. PCR were conducted over 32 cycles as follows (cycle 1 94°C, 1 min; cycle 2-31: 94°C, 30 sec, 58°C, 30 sec (or 55°C to amplify FIRE EGF1 domain), 68°C, 30 sec; cycle 32: 68°C, 5 min)

**3.3. Cloning of PCR Products**

PCR products were electrophoresed through 2% agarose gels containing ethidium bromide. The bands of interest were excised and the DNA was purified from the gel pieces using a 'Qiaex II Gel Extraction Kit' (Qiagen) according to the manufacturer's recommendations. The purified DNA from each PCR contained a *Bam*HI recognition site at its 5' end (indicated by the single underlined region in the sequences of all forward primers above). This *Bam*HI recognition site (and other restriction endonuclease recognition sites described below) was 'protected' during PCR by four non-specific flanking bases, indicated by double-underlining in Section 3.1. The DNA amplified by all primers of contructs (i)-(iv) (Section

3.1) contained a *Hind*III recognition sequence at its 3' end (indicated by single underline). All PCR products also contained a stop codon at their 3' ends (encoded in the reverse primer and shown in bold in section 3.1). Each of the purified PCR products was then cut with the appropriate restriction enzymes; ie; Constructs (i)-(iv) were cut with *Bam*HI and *Hind*III. Similarly, two vectors that had been chosen for bacterial expression, pMalp2 and pCaln, were treated with *Bam*HI and *Hind*III (both vectors). The PCR products were ligated into the appropriate vector and these plasmids were then used to transfect the *E.coli* strain, DH5α.

**4. Constructs and Immunisation Protocol**

FIRE and a control protein were expressed as FLAG tagged proteins on the surface of CHO cells. Briefly, primers (5' TAG TAG ACG CGT ATA TTA CAA ATG ATG AAT ATT (SEQ ID NO:20) and 5' TAG TAG ACG CGT TCA ATC ACT AAT AGT TCT GCT (SEQ ID NO:21)) were designed to amplify mouse FIRE without its leader sequence and to add adaptors that would allow subcloning into the pEF-BOS vector. The vector cDNA (http://www.wehi.edu.au/willson vectors) had been modified to contain the IL-3 leader sequence followed by the FLAG epitope and the cloning site that would allow the insertion of the FIRE cDNA. This construct resulted in the expression of FIRE proteins that contained the FLAG epitope at the N-terminus ie. extracellularly. Using FuGENE 6 Transfection Reagent (Boehringer Mannheim) CHO and 293T cells were co-transfected with the pEF-BOS-FIRE and a pCI-neo plasmid containing the neomycin phosphotransferase gene (kindly provided by Dr A Lew; Promega, Wisconsin) or pPGKpuroA (kindly provided by Leonie Gibson) at a ratio of 10:1. Transfectants were allowed to recover for 24 h before selection with 750 μg/ml G418 (Geneticin, GIBCO) commenced. FIRE-positive cells were stained with anti-FLAG mAb (IC7; kindly provide by Prof Nicola) followed by an anti-mouse-PE (Silenus) and isolated by sorting on MoFlow. After two rounds of this enrichment a pool of stable transfectants was established (Figure 1).

**5. Fc-Fusi n pr teins**

To produce soluble FIRE protein, the external portion of FIRE was amplified (using the following primers: 5' CGG GAT CCT CCT CAT GGG

GTA GAG CC (SEQ ID NO:22) and 5' CGG GTA CCA CCA TGG GAA GCA GGT GCC TTC TGC (SEQ ID NO:23)) then fused to the human IgG1 Fc domain and expressed in the Cigh vector (kindly provided by Dr A. Lew). The contruct was co-transfected with the pCI-neo plasmid into CHO cells. Transfectants were cloned by limiting dilution and clones that produced the Fc-fusion protein were selected using an anti-human Ig ELISA. Fc-FIRE was purified and enriched using an anti-human IgG agarose column (Sigma). The fusion protein was utilised in ELISA where an anti rat-HRP (Chemicon) antibody was used to detect sera that bound to Fc-FIRE.

**6. Immunisations and Monoclonal Ab Production**

Rats were immunised 4 times with 5-10 million CHO cells expressing FIRE-FLAG, then given a final boost four days prior to fusion. Hybridomas were produced by fusion of rat spleen cells with SP2/0 myeloma line using PEG 1500. Following HAT selection, wells containing hybridomas secreting specific monoclonal antibodies were identified by ELISA and FACS analysis of supernatants. Positive hybridomas were cloned by limiting dilution.

**7. Immunohistological Analysis**

Spleens were snap frozen in compound embedding medium (OCT) using liquid nitrogen. Sections (5 microns) were cut and fixed using ice-cold acetone. Fixed sections were first incubated with biotinylated FIRE mAb (3H7 and 6F12; 1 hr at room temperature), washed in PBS then incubated with ABC HRP kit (Vector Laboratories). The reaction was visualised using NovaRED (Vector Laboratories). Sections were counterstained with hematoxylin.

**8. FACS Analysis**

**8.1 Splenic DC and Macrophages**

Splenic DC were obtained as described previously (Vremec et al. 2000). To obtain splenic macrophages, spleens were mechanically disrupting by passing through a metal sieve. Cells were then resuspended in Nycodenz (1.091 g/cm$^3$) and the light density cells separated by density centrifugation. Irrelevant cells were then removed by incubation with anti-CD3 (KT3), anti-erythrocyte (TER119), and anti-B220 (RA36B2), anti-CD8 (53-6.7) anti-CD4

(GK1.5) followed by depletion using anti-rat Ig conjugated magnetic beads as per standard protocol (8). The macrophage enriched fraction was then stained with anti-M1/70 (CD11b) and anti-F4/80. Dead cells were excluded from analysis based on their uptake of propidium iodide (PI).

**8.2 Peripheral Blood Mononuclear Cells**

C57/B6 mice were bled by cardiac puncture into tubes containg heparin/PBS. Mononuclear cells were isolated by density centrifugation using lympholyte M (Cedarlane Laboratories). The light density cells were then incubated with mAb anti-CD3 (KT3), anti-Thy1.1 (T24/31.7), anti-Gr1 (RB68C5), anti-erythrocyte (TER119), and anti-B220 (RA36B2) and depleted using anti-Ig coupled Dynabeads (Vremec et al. 2000). The remaining mononuclear cells were stained and analysed for expression of FIRE.

**9. Cloning of Human FIRE**

The following 2 oligonucleotides 5' CACCTGCAGCTCTTCCATCT (SEQ ID NO:16) and 5' GAAAGTTTGCTTCTCAAAATCCA (SEQ ID NO:17), derived from sequences in the translated region of mouse FIRE, were used to amplify a fragment of human FIRE cDNA by low stringency PCR (annealing temperature: 50 degrees, Mg2+ concentration: 2.5mmol/l, 40 cycles) using target cDNA derived from both a human thymic preparation enriched in DCs and also from fresh and LPS activated human splenocytes. The resulting human FIRE sequence was 403 bp and had 83% homology with mouse FIRE at the DNA level. A 380 bp sub-fragment (isolated by PCR using the following primers 5' ggaagtagaacaccaggtttatca and 5' cctcttcctggcccacct) of the 403 bp human FIRE cDNA was then used to screen a commercial library (human bone marrow 5'-STRETCH cDNA library in lambda gt11, CLONTECH Laboratories, Palo Alto, CA) using conventional hybridisation methods. The resulting hybridising clone contained approximately 1482 bp of human FIRE. The remaining 5' human FIRE cDNA sequence was identified in clone RP11-1137G4 from the htgs database using a BLAST search. Primers 5' TGTCTCATTGCACCTCTTGGTTTCAT (SEQ ID NO:18) and 5' CCACAACAGCACCCACTGT (SEQ ID NO:19) were designed from sequences in clone RP11-1137G4 and used to amplify the 5' human FIRE cDNA using PCR.

## RESULTS

### 1. Cloning of mouse FIRE

RDA analysis was performed to compare gene expression in the myeloid-related $CD8^-Mac\text{-}1^+DC$ and the lymphoid-related $CD8^+Mac\text{-}1^-DC$. Results showed that as successive rounds of PCR and subtractive hybridisation occurred genes in common between the two populations were subtracted and not amplified (hence the background smear disappeared during progression from DP-1 (the first differential product) to DP-3 (the third differential product)). The bands which were observed in the DP-3 which corresponded to fragments of putative differentially expressed cDNA molecule (data not shown) were cloned and sequenced. House-keeping genes were detected only at very low frequency in these sequenced fragments suggesting that the RDA efficiently removed "common" sequences. To confirm that the bands from the DP-3 products were indeed differentially expressed in a minority population such as DC, several different approaches were taken. First, we generated a new "representation" from a separate source of RNA - this assures that any bias introduced in the first representation (used to generate the first RDA) would not be introduced in the reanalysis of differential expression. Using this approach, which is referred to as a "Virtual Northern", 9/11 gene fragments were found to be differentially expressed. This is a surprisingly high efficiency considering techniques such as RDA are prone to generating false positives, and indicates that this particular RDA successfully amplified differentially expressed genes. One of the DNA fragments was chosen for further analysis as it encoded a novel membrane protein, termed FIRE. Conventional Northern blot analysis confirmed data from Virtual Northerns and RT-PCR that FIRE is expressed at higher levels in myeloid DC than lymphoid DC.

Full length clones encoding this novel gene were obtained using conventional molecular biological techniques, involving the RDA generated fragments. The full length sequence of murine FIRE, showing both cDNA sequences and translated protein sequences, are shown in Figure 2.

FIRE encodes a 681 amino acid protein and analyses of the FIRE sequence shows that it is a novel member of the recently described EGF/TM7 superfamily (7), a comparison of the FIRE sequence with its distant relatives, the other members of the EGF/TM7 superfamily are shown in Figure 3. The

most famous member of the EGF/TM7 superfamily is F4/80 (mouse EMR-1) a molecule which is a marker of macrophages. Analysis of the FIRE sequence predicts a structure where there are two EGF domains in its extracellular region together with a "hinge" structure possessing a number of putative glycosylation sites. The protein then crosses the cell membrane seven times before a sizeable cytoplasmic domain of some 94 amino acids.

**2. Immunohistochemistry Analysis Results**

Four monoclonal antibodies (mAb) have been generated against mouse FIRE. Correspondingly, mAb could be generated against human FIRE.

Using the mAb against FIRE, it was determined that FIRE positive cells were predominantly present in the marginal zones and red pulp of mouse spleen. Such staining suggests that FIRE is expressed on DC present in the marginal zones and other antigen presenting cells (APC) such as macrophages that reside in the red-pulp.

**3. FACS Analysis Results**

In the mouse spleen, FIRE is predominantly expressed on the surface of DC (FIGURE 4A) and macrophages (FIGURE 4B and 4C). As suggested by the initial RDA results, FIRE is expressed more abundantly on the splenic DC that do not express CD4 and CD8, and to a lesser extent on the CD4+ DC and CD8+ DC (FIGURE 4A). Other cell types such as T and B cells do not appear to express FIRE, though it is possible that a very small population of either subsets express low levels of FIRE. A large proportion of blood mononuclear cells, including putative early DC, express high levels of FIRE (FIGURE 4E).

**4. Functional Data Results**

FIRE positive blood mononuclear cells could be the precursors of some CD8- lymphoid tissue DC. Peripheral blood mononuclear cells were isolated as described above. Cells were stained for FIRE expression, then sorted into population that were FIRE+ or FIRE- (MoFlow (Cytomation Inc., Fort Collins, CO). The sorted cells were incubated overnight at 37°C in medium alone or medium containing Fl3L, IL-4, GM-CSF, and TNF-α, then analysed for their expression of CD11c as a marker of DC. FIRE+ cells incubated in medium alone did not express high levels of CD11c (FIGURE 5A), but upon incubation with the cytokine cocktail, a large proportion of these cells

became CD11c+ (FIGURE 5B). Some CD11c+ cells were also generated from FIRE- cells, in medium alone (FIGURE5C) and medium plus cytokines (FIGURE 5D), though the proportion of these cells was not as high as when FIRE+ precursors were used. This data indicates that FIRE+ blood cells may be precursors of APC such as DC and macrophages.

**5. Cloning and expression of human FIRE**

The human FIRE DNA sequence was isolated as described in the materials and methods. Figure 6 shows the cDNA and amino acid sequences of human FIRE.

Human DC differentially express FIRE mRNA, where monocyte-derived DC express the highest level of FIRE whereas thymic DC express less message. Activation of DC via CD40 (using mAb against CD40) results in down-regulation of FIRE mRNA. Very little FIRE transcript could be detected by RT-PCR in T and B cells (see Table 1).

**Table 1. The expression of human FIRE assessed by RT-PCR**

| Cell type | human FIRE expression |
|---|---|
| Thymic preparation enriched in DC | + |
| Total splenocytes | + + + |
| Tonsillar $CD3^+$ T cells | - |
| Tonsillar $CD19^+$ B cells | - |
| Peripheral blood $CD14^+$ monocytes | + |
| Monocyte-derived DC | + + + |
| CD40-activated Monocyte-derived DC | + |
| $CD11b^-$ thymic DC | - |
| CD40-activated $CD11b^-$ thymic DC | - |
| $CD11b^+$ thymic DC | + |
| CD40-activated $CD11b^+$ thymic DC | - |

FIRE-specific primers used in RT-PCR were 5' ggaagtagaacaccaggtttatca (SEQ ID NO:24) and 5' cctcttcctggcccacct (SEQ ID NO:25).

FIRE is a marker for DC populations. The data obtained to date demonstrates that this molecule is expressed differentially or at much higher levels on the more "stimulatory" $CD8^-$ myeloid-related rather than the $CD8^+$ lymphoid-related dendritic cell populations, as they occur in the mouse spleen. Accordingly, it is believed that specific ligands such as monoclonal antibodies directed to this molecule will be useful reagents in identifying and particularly in purifying dendritic cells.

Publications referred to above are incorporated herein in their entirety by this reference.

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

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Claims:

1. An isolated polypeptide, the polypeptide comprising:-
   (i) an amino acid sequence as set out in SEQ ID NO:1, or
   (ii) an amino acid sequence having at least 50% identity to the amino acid sequence set out in SEQ ID NO:1, or
   (iii) a functional fragment of (i) or (ii).

2. An isolated polypeptide or peptide as claimed in claim 1, wherein the polypeptide or peptide has a sequence of at least 70%, more preferably at least 80% and most preferably at least 90% identity with the sequence shown in SEQ ID NO:1.

3. An isolated polypeptide, the polypeptide comprising:-
   (i) an amino acid sequence as set out in SEQ ID NO:2, or
   (ii) an amino acid sequence having at least 50% identity to the amino acid sequence set out in SEQ ID NO:2, or
   (iii) a functional fragment of (i) or (ii).

4. An isolated polypeptide or peptide as claimed in claim 3, wherein the polypeptide or peptide has a sequence of at least 70%, more preferably at least 80% and most preferably at least 90% identity with the sequence shown in SEQ ID NO:2.

5. An isolated ligand, the ligand being interactive with the polypeptide or peptide of any one of claims 1 to 4.

6. An isolated ligand as claimed in claim 5, wherein the ligand is an antibody or the binding portion thereof.

7. An isolated nucleic acid molecule, the nucleic acid molecule encoding a polypeptide as claimed in any one of claims 1 to 4.

8. An isolated nucleic acid molecule, the nucleic acid molecule comprising:-

(i) a sequence as set out in SEQ ID NO:3, or

(ii) a sequence having at least 60% identity to the sequence set out in SEQ ID NO:3, or

(iii) a sequence which hybridises to the sequence set out in SEQ ID NO:3 under stringent conditions, or

(iv) a sequence encoding a functional analogue of a polypeptide as set out in SEQ ID NO:1.

9. An isolated nucleic acid molecule as claimed in claim 8, wherein the nucleic acid molecule comprises a sequence of at least 70%, more preferably at least 80% and most preferably at least 90% identity with the sequence shown in SEQ ID NO:3.

10. An isolated nucleic acid molecule, the nucleic acid molecule comprising:-

(i) a sequence as set out in SEQ ID NO:4, or

(ii) a sequence having at least 60% identity to the sequence set out in SEQ ID NO:4, or

(iii) a sequence which hybridises to the sequence set out in SEQ ID NO:4 under stringent conditions, or

(iv) a sequence encoding a functional analogue of a polypeptide as set out in SEQ ID NO:2.

11. An isolated nucleic acid molecule as claimed in claim 10, wherein the nucleic acid molecule comprises a sequence of at least 70%, more preferably at least 80% and most preferably at least 90% identity with the sequence shown in SEQ ID NO:4.

12. An isolated nucleic acid molecule, the nucleic acid molecule encoding the binding region of a ligand as claimed in claim 5 or claim 6.

13. A composition for use in raising or lowering an immune response in a subject, the composition comprising a ligand as claimed in claim 5 or claim 6 and an antigen and optionally a carrier and/or adjuvant.

14. A composition as claimed in claim 13, wherein the antigen is associated with the ligand.

15. A composition as claimed in claim 13, wherein the antigen is conjugated to the ligand.

16. A composition for use in raising or lowering an immune response in a subject, the composition comprising a nucleic acid molecule and a carrier, the nucleic acid molecule comprising a first sequence encoding a ligand as claimed in claim 5 or claim 6 and a second sequence encoding an antigen.

17. A method of screening a putative compound for immunological regulatory activity, the method comprising reacting the compound with a polypeptide or peptide as claimed in any one of claims 1 to 4 and measuring interaction between the compound and the polypeptide or peptide.

18. A method of isolating an antigen presenting cell from a biological sample, the method comprising contacting the biological sample with a ligand as claimed in claim 5 or claim 6 such that a complex is formed between the ligand and the antigen presenting cell.

19. A method as claimed in claim 18 wherein the ligand is immobilised on a solid support.

20. A method of immunising a subject, the method comprising

(i) isolating antigen presenting cells from a fluid sample obtained from the subject, wherein the isolation involves contacting the fluid sample with a ligand as claimed in claim 5 or claim 6;

(ii) exposing the cells isolated from step (i) to an antigen; and

(iii) reintroducing the cells from step (ii) into the subject.

21. A method as claimed in claim 20, in which the method comprises the further step of growing the antigen presenting cells *in vitro* after step (i).

22 A method of immunising a subject, the method comprising:

(i) isolating precursor cells from a fluid sample obtained from the subject, wherein the isolation involves contacting the fluid sample with a ligand as claimed in claim 5 or claim 6

(ii) growing the cells isolated from step (i) *in vitro* such that they mature and differentiate to become antigen presenting cells

(iii) exposing the cells obtained in step (ii) to an antigen

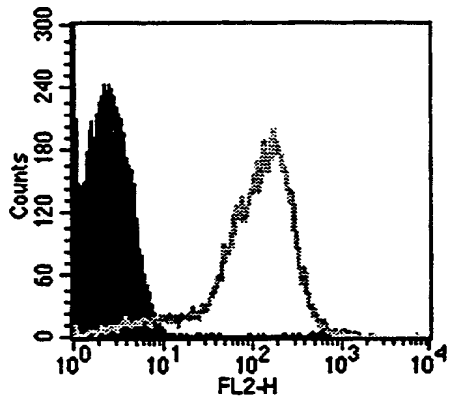
(iv) reintroducing the cells from step (iii) into the subject

23. A method of modulating an immune response in a subject, the method comprising administering to the subject a ligand as claimed in claim 5 or claim 6 such that the ligand binds to and inhibits the function of an antigen presenting cell.

24. A method as claimed in claim 23 wherein the antigen presenting cell is a myeloid dendritic cell.

25. A method as claimed in claim 23 or claim 24 in which the method further comprises the step of administering an antigen to the subject.

26. A method as claimed in claim 25 in which the antigen is administered after administration of the ligand.




Anti-FLAG ⟶

**Figure 1**

```
                10                  30                  50                  70                  90
                .                   .                   .                   .                   .
ACCACTGCTTCATTGCTGCTGAGAATGTTCCAGGCTGAGTGAGAAGTAAAAATTCATCATCTCTGAAGAACTCTTACCCAGCCCTGTTGA
              110                 130                 150                 170

                                    M  G  S  R  C  L  L  H  A  S  V  P  G  M  L  L  I  W      18
1   AGAAATTCCCAGAATGTTGATGGGAGCAACTAGAGATATGGGAAGCAGGTGCCTTCTGCATGCCTCAGTTCCTGGAATGCTGCTTATCTG
          190                 210                 230                 250                 270

                S  I  L  Q  M  M  N  I  S  A  S  C  P  Q  C  N  E  N  A  S  C  F  N  S  T  H  C  V  C  K      48
19  GTCAATATTACAAATGATGAATATTTCAGCTTCCTGTCCCCAGTGCAATGAAAATGCCAGCTGCTTCAACAGCACCCACTGTGTTTGTAA
              290                 310                 330                 350

                E  G  F  W  T  G  S  E  N  R  R  I  I  E  P  H  E  K  C  Q  D  I  N  E  C  L  L  K  E  L      78
49  AGAAGGATTCTGGACGGGCTCTGAGAATAGAAGAATTATTGAGCCCCATGAGAAATGTCAAGATATTAATGAGTGTCTACTGAAAGAATT
              370                 390                 410                 430                 450

                V  C  K  D  V  S  Y  C  R  N  K  I  G  T  Y  I  C  S  C  V  V  K  Y  P  L  F  N  W  V  A      108
79  GGTATGCAAGGATGTGTCGTACTGCAGAAATAAAATTGGGACTTACATATGCAGCTGTGTAGTAAAATATCCTTTGTTCAACTGGGTAGC
              470                 490                 510                 530

                G  I  I  N  I  D  H  P  D  D  C  Y  V  V  N  K  S  K  N  T  G  S  K  T  H  T  L  G  V  L  S  E      138
109 TGGCATTATTAATATTGATCACCCTGATTGTTATGTGAACAAGAGCAAGAATACAGGATCAAAAACACATACTTTGGGAGTACTGAGTGA
              550                 570                 590                 610                 630

                F  K  S  K  E  E  V  A  K  G  A  T  K  L  L  R  K  V  E  H  H  I  L  N  E  N  S  D  I  P      168
139 ATTTAAATCCAAAGAGGAGGTTGCAAAAGGAGCTACCAAGTTACTTCGCAAAGTGGAACATCACATCTTGAATGAAAACTCAGATATACC
              650                 670                 690                 710

                K  K  D  E  N  P  L  L  D  I  V  Y  Y  E  T  K  R  C  K  T  M  T  L  L  E  A  G  N  N  T  M      198
169 AAAAAAGGATGAAAATCCTTTATTGGATATAGTGTATGAAACTAAGAGGTGCAAGACGATGACTCTTCTAGAAGCTGGCAACAACACAAT
              730                 750                 770                 790                 810

                K  V  D  C  T  S  G  F  K  E  H  N  S  G  G  E  T  A  V  A  F  I  A  Y  K  S  L  G  N  L      228
199 GAAGGTTGACTGCACTAGTGGTTTCAAAGAGCACACAACAGTGGAGGTGAAACTGCAGTGGCTTTCATTGCATATAAGTCTCTTGGGAATCT
              830                 850                 870                 890

                L  N  G  S  F  S  N  E  E  G  F  Q  E  V  T  L  N  S  H  I  V  S  G  A  I  R  S  E  V      258
229 TCTAAATGGTTCCTTTTTTAGTAATGAAGAAGGGTTTCAGGAAGTGACACTGAACTCTCACATCGTTAGTGGAGCCATTCGCTCAGAGGT
```

**Figur 2**

```
910                930                950                970                990
259 K P V L S E P V L L T L Q N I Q P I D S R A E H L C V H W E 288
    CAACCTGTCCTCTCTGAACCTGTACTCCTGACTTTACAAAATATTCAGCCCATTGACTCAAGAGCAGAACATCTCTGTGTCCATTGGGA
          1010               1030               1050               1070
289 G S E E G G S W S T K G C S H V Y T N N S Y T I C K C F H L 318
    AGGATCAGAGGAAGGGGGGAGCTGGTCTACCAAAGGATGCTCTCACGTGTACACCAATAATTCCTACACCATTTGCAAGTGTTTCCACCT
          1090               1110               1130               1150               1170
319 S S F A V L M A L P H E E D G V L S A L S V I T Y V G L S L 348
    GTCCAGCTTTGCTGTGCTCATGGCTCTACCCCATGAGGAGGATGGTGTGCTTTCTGCACTCTCTGTGATCACCTATGTGGGACTGAGTCT
          1190               1210               1230               1250
349 S L L C L F L A A I T F L L C R P I Q N T S T T L H L Q L S 378
    TTCTCTCTTGTGCCTATTTCTGGCGGCCATCACTTTTCTCCTGTGCCGACCCATTCAGAATACCAGCACGACACTCCACCTGCAGCTCTC
          1270               1290               1310               1330               1350
379 I C L F L A D L L L F L T G I N R T K P K V L C S I I A G M L 408
    CATCTGCCTTTTCCTGGCTGACCTCCTCTTCCTCACAGGCATCAACAGAACTAAGCCTAAGGTGCTGTGCTCCATCATAGCGGGGATGTT
          1370               1390               1410               1430
409 H Y L Y L A S F M W M F L E G L H L F L T V S N L K V A N Y 438
    GCACTACCTCTACTTGGCTTCCTTCATGTGGATGTTTCTGGAAGGGCTACATCTTTTTCTCACTGTGAGCAATCTCAAAGTGGCCAACTA
          1450               1470               1490               1510               1530
439 S N S G R F K K R F M Y P V G Y G L P A F I V A V S A I A G 468
    CAGCAACTCAGGCAGATTCAAGAAGAGGTTCATGTATCCTGTAGGATATGGGCTTCCTGCTTTTATTGTTGCTGTATCTGCAATAGCTGG
          1550               1570               1590               1610
469 H K N Y G T H N H C W L S L H R G F I W S F L G P A A I I 498
    CCACAAGAATTATGGAACACACAACCACTGCTGGCTCAGCCTTCATCGAGGATTCATCTGGAGCTTCTTGGGGCCAGCGGCAGCCATTAT
          1630               1650               1670               1690               1710
499 L I N L V F Y F L I I W I L R S K L S S L N K E V S T L Q D 528
    CTTGATAAACCTGGTGTTCTACTTTCTAATAATATGGATTTTGAGAAGCAAACTTTCTTCTCAATAAGAAGTTTCTACACTTCAAGA
          1730               1750               1770               1790
529 T K V M T F K A I V Q L F V L G C S W G I G L F I F I E V G 558
    CACAAAGGTTATGACATTTAAAGCCATTGTCCAGTTATTTGTGTTGGGATGTTCTTGGGGCATTGGCTTGTTTATTTTCATTGAAGTTGG
```

**Figure 2 (continued)**

```
                1810                1830                1850                1870                1890
                .                   .                   .                   .                   .
559  K T V R L I V A Y L F T I I N V L Q G V L I F M V H C L L N  588
     GAAGACAGTGAGACTGATCGTTGCCTATCTGTTCACCATCAATGTCCTGCAGGGTGTTTTGATATTTATGGTACATTGTCTGCTTAA
                1910                1930                1950                1970

589  R Q V R M E Y K K W F H R L R K E V E S S E S T E V S H S T T  618
     TCGCCAGGTGCGGATGGAATATAAGAAGTGGTTTCATAGACTGCGGAAGGAAGTTGAAAGTGAAAGCACTGAAGTGTCTCATTCTACTAC
                1990                2010                2030                2050                2070

619  H T K M G L S L N L E N F C P T G N L H D P S D S I L P S T  648
     TCACACAAAAATGGGTCTTTCTCTGAACCTGGAAAATTTCTGCCCAACAGGAAACCTCCATGATCCTTCTGACTCCATCCTTCCAAGTAC
                2090                2110                2130                2150

649  E V A G V Y L S T P R S H M G A E D V N S G T H A Y W S R T  678
     TGAAGTAGCAGGTGTATATCTAAGCACACCCAGGTCTCACATGGGTGCTGAGGATGTGAACTCAGGTACTCACGCTTACTGGAGCAGAAC
                2170                2190                2210                2230                2250

679  I S D *  681
     TATTAGTGATTGAATCAGCTCCTTCCCCCAAGCCTCTTACAGTACATTTTAACTTGTACTGTGCCATGCACATGAAGCTATAATTGCTAG
                2270                2290                2310                2330

     TCTGGTAAAACAACTGTTGCATATTCCATGATCATTTCATTTTATCTCTACTTGCAAAAGTTAGCTTTCTTTTTATATCATTTTTATTTC
                2350                2370                2390                2410                2430

     TCTTTCTTTGTTTATATAGCTTCAGTTGAGTGGGTTTCTAGTCTTAATGTTCTAGATCACTATTTTCTTTTTCAGTTAACCTTTATTG
                2450                2470                2490                2510

     GTATTTAGTTCCTGTGTAGTGTATACCACTGGAATATTTTTATTTCTTTAATTTTGAGGTTAAAATATAGTTACATCATTTTTTCCTTTTT
                2530                2550                2570                2590                2610

     TTCTTTCCCACAATCCTCCTGTATACTTTTTCCCTGGTGTCTATTTTATTGTTTCTACATGCATATATATTTTATGCAAAACATATATAT
                2630                2650                2670                2690

     GTATAAATATAAATATATATTCTTATATGCATGAAAACCATCTACTTCATCCAAATAATGTTCCTTCTATGTATGTTTTCAGGACAGGGA
                2710                2730                2750                2770                2790

     CAACAATAGCTATGGTAGCATGGCAGGGGAAAGCCCACAGGACCTCAGCCTTATACAAAGAATCAGAGGCAACTGAGGAGTGCTGAGTTG
                2810                2830                2850                2870

     AAGGAATTGTCTTACCCAGGGGAGGGCACATTAATTGGTTATCTAATACAAAATGTTCAGCCCCAAACTGTTAAGATAAAAGCCTATAT
```

**Figure 2 (continued)**

```
2890        2910        2930        2950        2970
.           .           .           .           .
GCATCTTAGGAAGTATCTACCTTGATACACCTTTATTGGAATATCATCCACATGTTTATTGTGTGTTCTGAAGAGGGTCTGTTGAATTTC
        2990        3010        3030        3050
        .           .           .           .           .
TAAGGGTTGATCAGTTTAATTGTGCCATTTTATATTCAGGGTGTTTGGCTTTGTTGTAGTGAATAATGCTATATTTCCCTGTATGTGTCA
    3070        3090        3110        3130        3150
    .           .           .           .           .           .
TCTTTGACTGTTATTTTTTCCTGGCGATACTTTATTCAACAAGAACCTAGAGCCTTGGTTTATTACTTTTTCTTCCATAGAAAAACTATT
            3170        3190        3210        3230
            .           .           .           .           .
TGTCTTCCAGGATTAGATATGATCAATATTTCTTATATGCATGTATCAAATATCATGATGAAATATATTACTGTGTATAATTAATAACTG
        3250
        .
GCAATAAAGTCCAAGGGA
```

**Figure 2 (continued)**

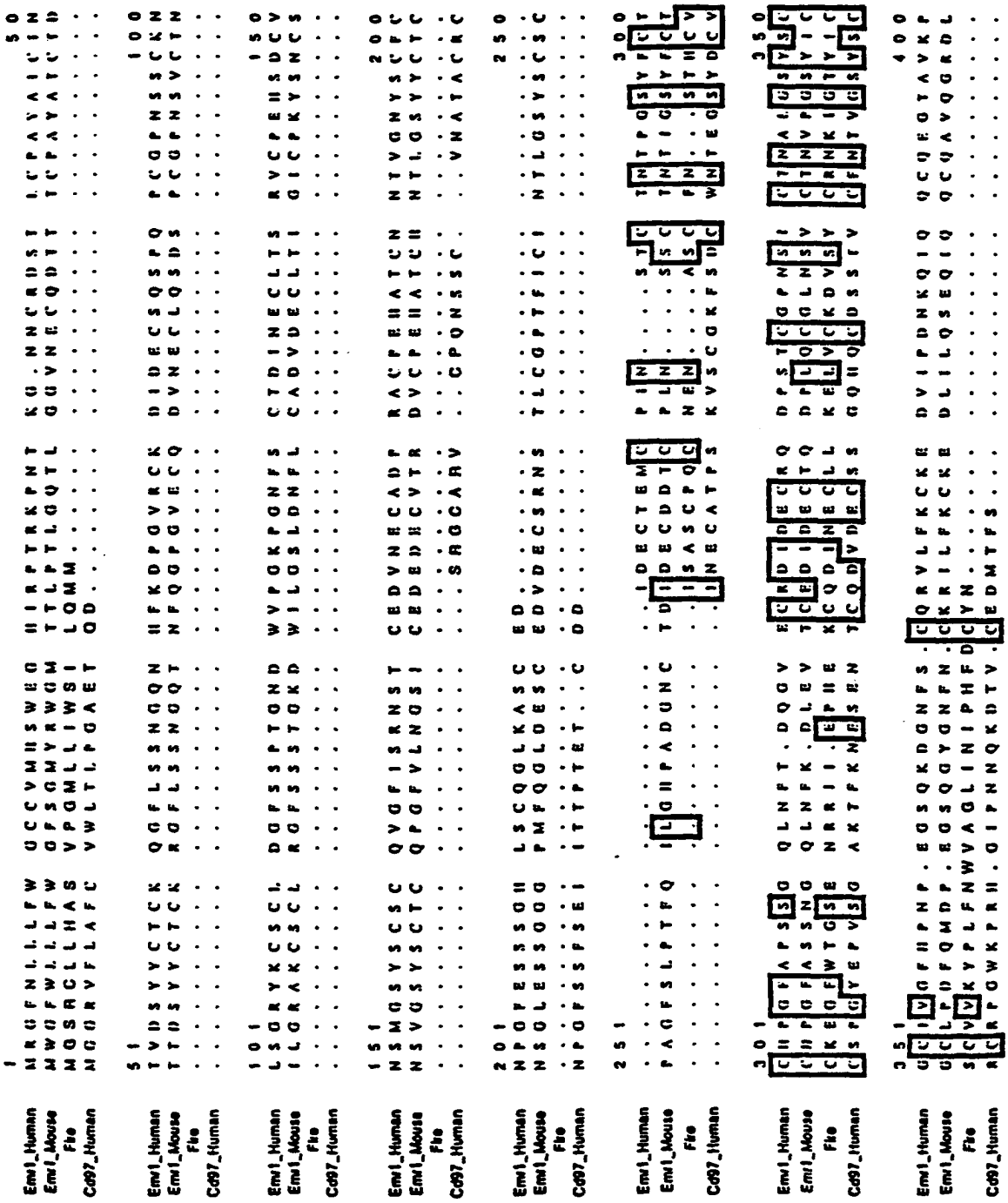
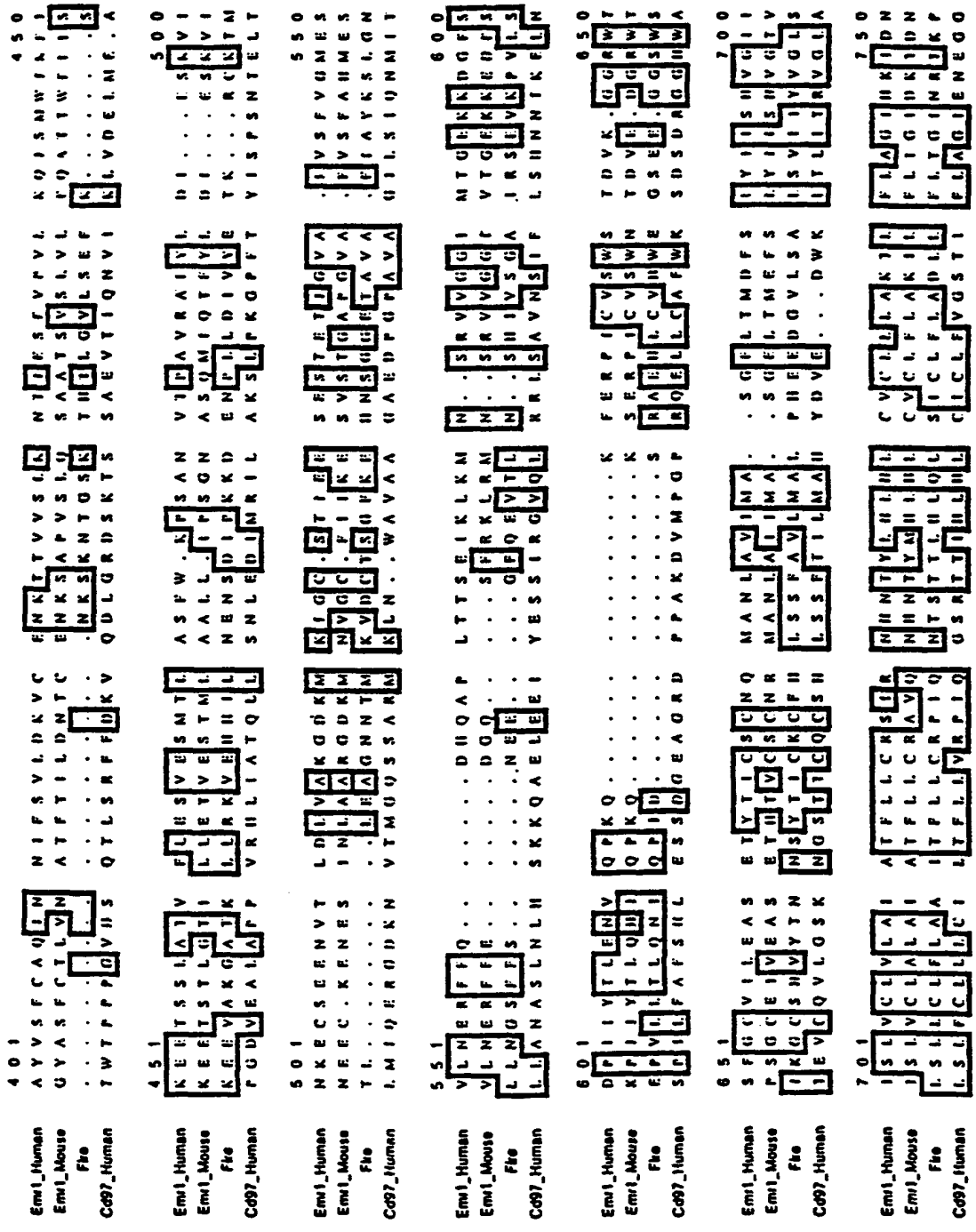


Figure 3



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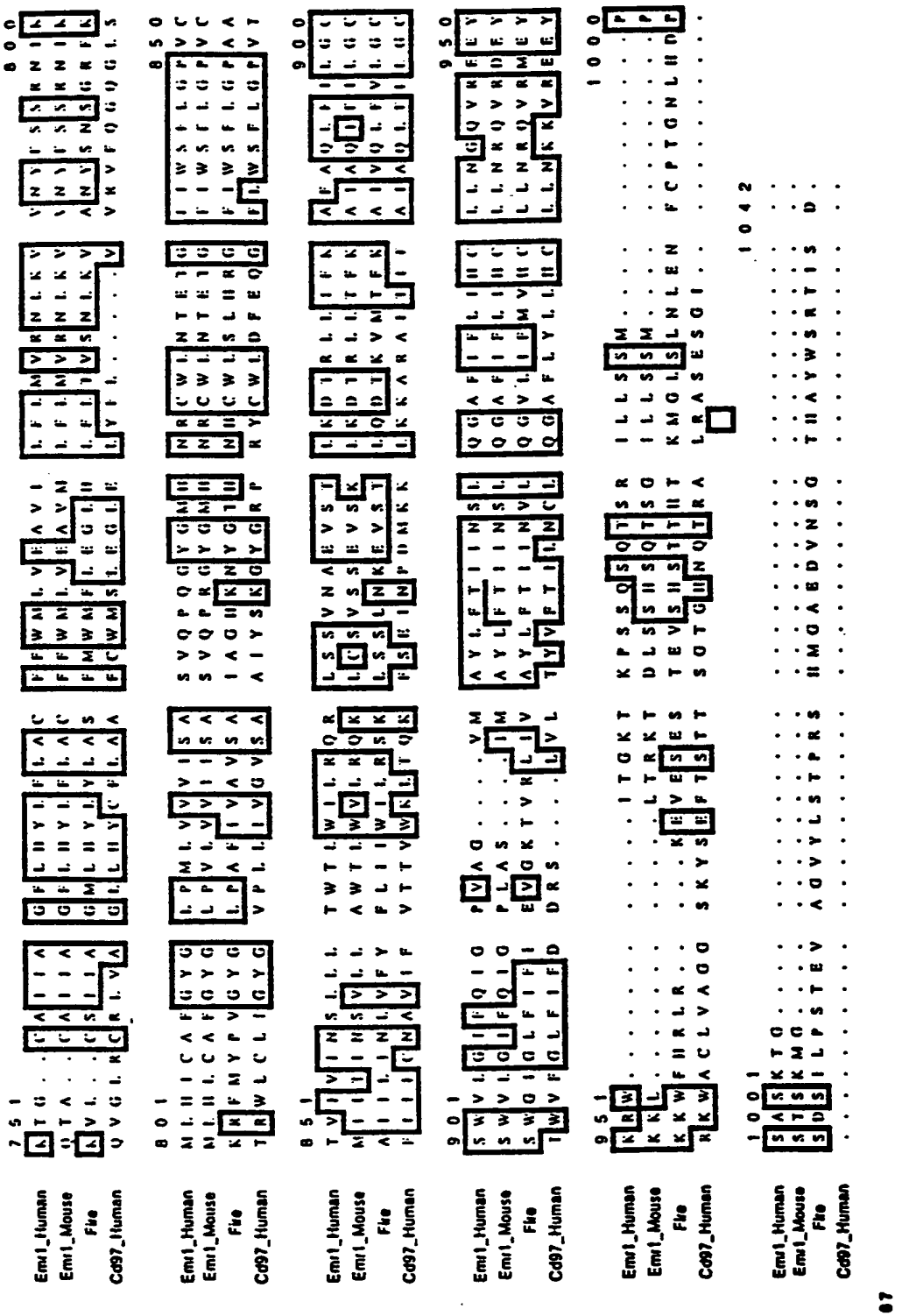
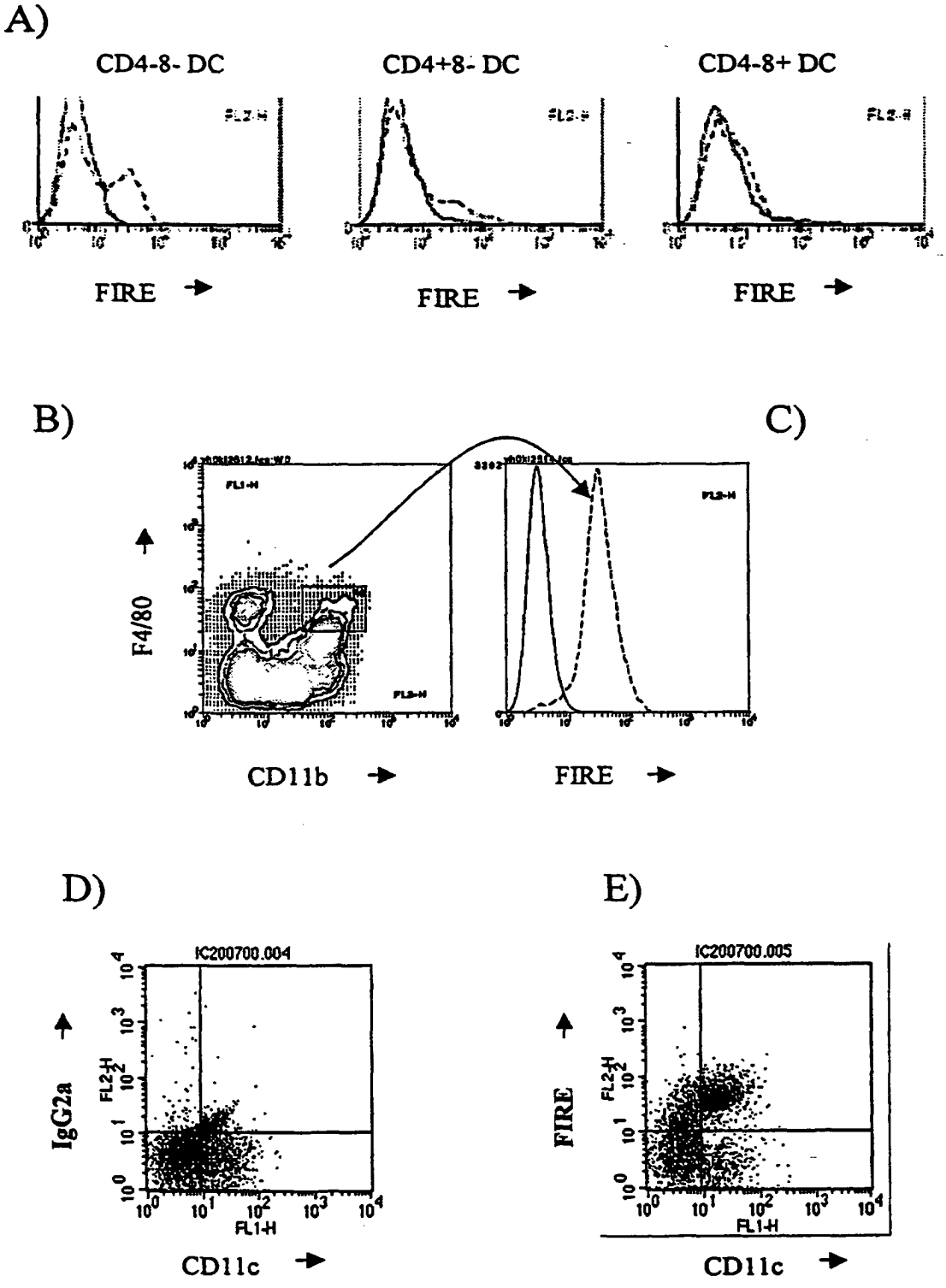


Figure 3 (continued)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CD4+8- DC
CD4-8+ DC
FIRE
FIRE
FIRE
B)
C)
F4/80
CD11b
FIRE
D)
E)
IC200700.004
IC200700.005
IgG2a
FIRE
CD11c
CD11c
FL1-H
FL2-H

**Figure 4**

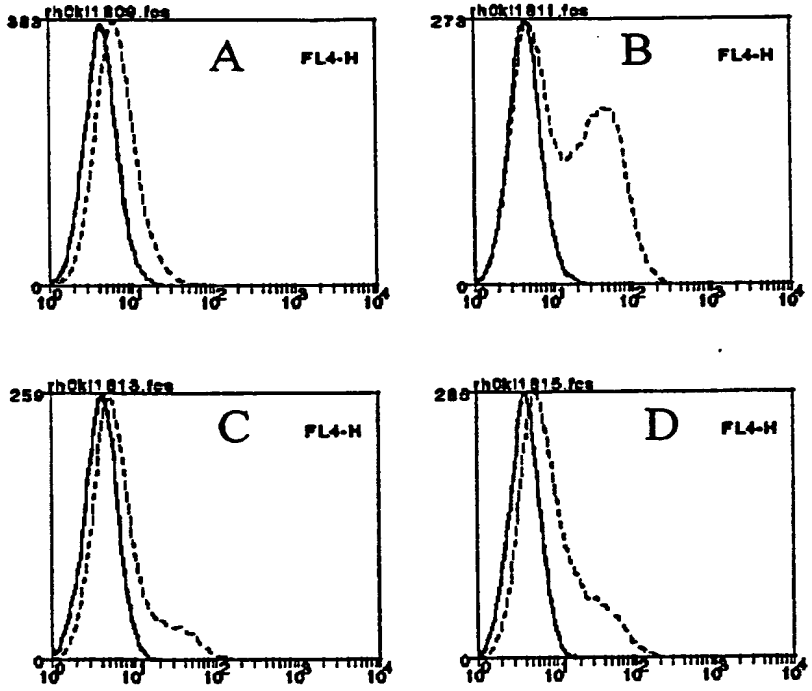


**Figure 5**

```
                A  S  C  P  P  C  P  K  Y  A  S  C  H  N  S  T  H  C  T  C  E  D  G  F  R  A  R  S  G  R
    1   GCTTCCTGTCCTCCATGCCCTAAATATGCCAGCTGCCACACAACAGCACCCACTGTACTTGTGAAGATGGCTTTCGGGCCAGGTCTGGCAGG   90
                T  Y  F  H  D  S  S  E  K  C  E  D  I  N  E  C  E  T  G  L  A  K  C  K  Y  K  A  Y  C  R
   91   ACATACTTTCATGATTCCTCTGAGAAGTGTGAAGATATTAATGAATGTGAAACCGGGCTGGCAAAGTGCAAGTATAAAGCATATTGTAGG   180
                N  K  V  G  G  Y  I  C  S  C  L  V  K  Y  T  L  F  N  F  L  A  G  I  I  D  Y  D  H  P  D
  181   AATAAAGTTGGAGGTTACATCTGTAGCTGTTTGGTAAAATATACTTTATTCAACTTTCTGGCTGGTATTATAGATTATGATCATCCGGAT   270
                C  Y  E  N  N  S  Q  G  G  T  T  Q  S  N  V  D  I  W  E  N  L  R  R  N  G  S  R  E  D  F  A
  271   TGTTACGAGAACAATAGTCAAGGGACGACACAGTCAAACGTGGATATTTGGGAAAATCTGAGAAGAAATGGAAGCAGAGAGGACTTTGCA   360
                R  R  A  T  Q  L  I  Q  S  V  E  L  S  I  W  N  A  S  F  A  S  P  G  K  G  Q  I  S  E  F
  361   AGAAGGGCTACTCAACTAATTCAAAGCGTGGAGTTGAGCATCTGGAATGCGAGTTTTGCTTCTCCAGGAAAGGGTCAAATTTCTGAATTT   450
                D  I  V  Y  E  T  K  R  C  N  E  T  R  E  N  A  F  L  E  A  G  N  N  T  M  D  I  N  C  A
  451   GATATAGTCTATGAAACCAAGAGGTGCAATGAGACAAGGGAGAATGCTTTTCTGGAAGCTGGAAATAACACCATGGATATCAACTGTGCT   540
                D  A  L  K  G  N  L  R  E  S  T  A  V  A  L  I  T  Y  Q  S  L  G  D  I  L  N  A  S  F  F
  541   GATGCTTTAAAAGGAAACCTAAGAGAGAGCACTGCAGTTGCCCTAATCACTTATCAATCTCTTGGGGATATTCTGAATGCATCCTTTTTT   630
                S  K  R  K  G  M  Q  E  V  K  L  N  S  Y  V  V  S  G  T  V  G  L  K  E  K  I  S  L  S  E
  631   AGTAAACGAAAAGGGATGCAGGAAGTAAAACTGAACTCTTACGTTGTGAGCGGCACCGTCGGTTTGAAGGAAAAAATTTCCCTCTCTGAA   720
                P  V  F  L  T  F  R  H  N  Q  P  G  D  K  R  T  K  H  I  C  V  Y  W  E  G  S  E  G  G  R
  721   CCTGTGTTCCTGACTTTTCGCCATAATCAGCCTGGTGACAAGAGAACAAAACATATCTGTGTCTACTGGGAGGGATCAGAGGGAGGCCGC   810
                W  S  T  E  G  C  S  H  V  H  S  N  G  S  Y  T  K  C  K  C  F  H  L  S  S  F  A  V  L  V
  811   TGGTCCACGGAGGGCTGCTCTCATGTGCACAGCAACGGTTCTTACACCAAATGCAAGTGCTTCCATCTGTCCAGCTTTGCCGTCCTCGTG   900
                A  L  A  P  K  E  D  P  V  L  T  V  I  T  Q  V  G  L  T  I  S  L  L  C  L  F  L  A  I  L
  901   GCTCTTGCCCCCAAGGAGGACCCTGTGCTGACCGTGATCACCCAGGTGGGGCTGACCATCTCYCTGCTGTGCCTCTTCCTGGCCATCCTC   990
                T  F  L  L  C  R  P  I  Q  N  T  S  T  S  L  H  L  E  L  S  L  C  L  F  L  A  H  L  L  F
  991   ACCTTCCTCCTGTGCCGGCCCATCCAGAACACCAGCACCTCCCTCCATCTAGAGCTCTCCCTCTGCCTCTTCCTGGCCCACCTCCTGTTC  1080
                L  T  G  I  N  R  T  E  P  E  V  L  C  S  I  I  A  G  L  L  H  F  L  Y  L  A  C  F  T  W
 1081   CTGACGGGCATCAACAGAACTGAGCCTGAGGTGCTGTGCTCCATCATTGCAGGGCTGCTCACTTCCTCTACCTGGCTTGCTTCACCTGG  1170
                M  L  L  E  G  L  H  L  F  L  T  V  R  N  L  K  V  A  N  Y  T  S  T  G  R  F  K  K  R  F
 1171   ATGCTCCTGGAAGGGCTGCACCTCTTCCTCACCGTCAGGAACCTCAAGGTGGCCAACTACACCAGCACGGGCAGATTCAAGAAGAGGTTC  1260
                M  Y  P  V  G  Y  G  I  P  A  V  I  I  A  V  S  A  I  V  G  P  Q  N  Y  G  T  F  T  H  C
 1261   ATGTACCCTGTAGGCTACGGGATCCCAGCTGTGATTATTGCTGTGTCAGCAATAGTTGGACCCCAGAATTATGGAACATTTACTCACTGT  1350
                W  L  K  L  D  K  G  F  I  W  S  F  M  G  P  V  A  V  I  I  L  I  N  L  V  F  Y  F  Q  V
 1351   TGGCTCAAGCTTGATAAAGGATTCATCTGGAGCTTCATGGGGCCAGTAGCAGTCATTATCTTGATAAACCTGGTGTTCTACTTCCAAGTT  1440
                L  W  I  L  R  S  K  L  S  S  L  N  K  E  V  S  T  I  Q  D  T  R  V  M  T  F  K  A  I  S
 1441   CTGTGGATTTTGAGAAGCAAACTTTCCCTCCCTCAATAAAGAAGTTTCCACCATTCAGGACACCAGAGTCATGACATTTAAAGCCATTTCT  1530
                Q  L  F  I  L  G  C  S  W  G  L  G  F  F  M  V  E  E  V  G  K  T  I  G  S  I  I  A  Y  S
 1531   CAGCTATTTATCCTGGGCTGTTCTTGGGGCCTTGGTTTTTTTATGGTTGAAGAAGTAGGGAAGACGATTGGATCAATCATTGCATACTCA  1620
```

**Figure 6**

```
                F  T  I  I  N  T  L  Q  G  V  L  L  F  V  V  H  C  L  L  N  R  Q  V  R  M  E  Y  K  K  W
1621  TTCACCATCATCAACACCCTTCAGGGAGTGTTGCTCTTTGTGGTACACTGTCTCCTTAATCGCCAGGTTCGAATGGAATATAAAAAGTGG  1710
                F  S  G  M  R  K  G  V  E  T  E  S  T  E  M  S  R  S  T  T  Q  T  K  K  T  E  E  V  G  K  S
1711  TTTAGTGGGATGCGGAAAGGGGTAGAAACTGAAAGCACTGAGATGTCTCGCTCTACTACCCAAACCAAACCAAAACGGAAGAAGTGGGGAAGTCC  1800
                S  E  I  F  H  K  G  G  T  A  S  S  S  A  E  S  T  K  Q  P  Q  P  Q  V  H  L  V  S  A  A
1801  TCAGAAATCTTTCATAAAGGAGGCACTGCATCATCATCTGCAGAGTCAACCAAGCAACCGCAGCCACAGGTTCATCTCGTCTCTGCTGCT  1890
                W  L  K  M  N  *
1891  TGGCTAAAGATGAACTGACCTGGCAAGTGCCATGGCAATGACCCGGAAGTTACCGCTCCTTTCCGTTTGTCTACAGCGCCCCTGTGGTCA  1980
1981  CACATAGATTGGACAAATGCCACTATTTCTAGCTTTCCTGTGAAAAGTCTAGGCTCATTCACCTATTTTGGCTTTTTATGTTCATAGAAA  2070
2071  GAACAAGACATTTGGGAGAATTCTTAGATCCAGAGTCCAGTAGTGTGGCACGTGCAATGAAGTGTCGGAAGGATGCATTTTAAAGATGGC  2160
2161  GGGCGGGAGAAGTGGATTTTTCTTCTTGCAGCTACTGCCACCTTGCCAGAAACTTCACTAACTGGCATCTGGRATTCAGCTCATAGTTCC  2250
2251  CTTTCTGGCCTCTCTGCTGTATTTTATGCTCCCAAAGATCTTACATTAACACTCCACATTCACATAATTCAACAATTTCATATGGATCA  2340
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2791  CACCAATC  2798
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**Figure 6 (continued)**

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 1               5                  10                  15

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            20                  25                  30

Cys Asn Glu Asn Ala Ser Cys Phe Asn Ser Thr His Cys Val Cys Lys
        35                  40                  45

Glu Gly Phe Trp Thr Gly Ser Glu Asn Arg Arg Ile Ile Glu Pro His
    50                  55                  60

Glu Lys Cys Gln Asp Ile Asn Glu Cys Leu Leu Lys Glu Leu Val Cys
65                  70                  75                  80

Lys Asp Val Ser Tyr Cys Arg Asn Lys Ile Gly Thr Tyr Ile Cys Ser
                85                  90                  95

Cys Val Val Lys Tyr Pro Leu Phe Asn Trp Val Ala Gly Ile Ile Asn
            100                 105                 110

Ile Asp His Pro Asp Cys Tyr Val Asn Lys Ser Lys Asn Thr Gly Ser
        115                 120                 125

Lys Thr His Thr Leu Gly Val Leu Ser Glu Phe Lys Ser Lys Glu Glu
    130                 135                 140

Val Ala Lys Gly Ala Thr Lys Leu Leu Arg Lys Val Glu His His Ile
145                 150                 155                 160

Leu Asn Glu Asn Ser Asp Ile Pro Lys Lys Asp Glu Asn Pro Leu Leu
                165                 170                 175

Asp Ile Val Tyr Glu Thr Lys Arg Cys Lys Thr Met Thr Leu Leu Glu
            180                 185                 190

Ala Gly Asn Asn Thr Met Lys Val Asp Cys Thr Ser Gly Phe Lys Glu
        195                 200                 205

His Asn Ser Gly Gly Glu Thr Ala Val Ala Phe Ile Ala Tyr Lys Ser
```

```
    210                 215                 220

Leu Gly Asn Leu Leu Asn Gly Ser Phe Phe Ser Asn Glu Glu Gly Phe
225                 230                 235                 240

Gln Glu Val Thr Leu Asn Ser His Ile Val Ser Gly Ala Ile Arg Ser
                245                 250                 255

Glu Val Lys Pro Val Leu Ser Glu Pro Val Leu Leu Thr Leu Gln Asn
            260                 265                 270

Ile Gln Pro Ile Asp Ser Arg Ala Glu His Leu Cys Val His Trp Glu
        275                 280                 285

Gly Ser Glu Glu Gly Gly Ser Trp Ser Thr Lys Gly Cys Ser His Val
    290                 295                 300

Tyr Thr Asn Asn Ser Tyr Thr Ile Cys Lys Cys Phe His Leu Ser Ser
305                 310                 315                 320

Phe Ala Val Leu Met Ala Leu Pro His Glu Glu Asp Gly Val Leu Ser
                325                 330                 335

Ala Leu Ser Val Ile Thr Tyr Val Gly Leu Ser Leu Ser Leu Leu Cys
            340                 345                 350

Leu Phe Leu Ala Ala Ile Thr Phe Leu Leu Cys Arg Pro Ile Gln Asn
        355                 360                 365

Thr Ser Thr Thr Leu His Leu Gln Leu Ser Ile Cys Leu Phe Leu Ala
    370                 375                 380

Asp Leu Leu Phe Leu Thr Gly Ile Asn Arg Thr Lys Pro Lys Val Leu
385                 390                 395                 400

Cys Ser Ile Ile Ala Gly Met Leu His Tyr Leu Tyr Leu Ala Ser Phe
                405                 410                 415

Met Trp Met Phe Leu Glu Gly Leu His Leu Phe Leu Thr Val Ser Asn
            420                 425                 430

Leu Lys Val Ala Asn Tyr Ser Asn Ser Gly Arg Phe Lys Lys Arg Phe
        435                 440                 445

Met Tyr Pro Val Gly Tyr Gly Leu Pro Ala Phe Ile Val Ala Val Ser
    450                 455                 460

Ala Ile Ala Gly His Lys Asn Tyr Gly Thr His Asn His Cys Trp Leu
465                 470                 475                 480

Ser Leu His Arg Gly Phe Ile Trp Ser Phe Leu Gly Pro Ala Ala Ala
                485                 490                 495

Ile Ile Leu Ile Asn Leu Val Phe Tyr Phe Leu Ile Ile Trp Ile Leu
            500                 505                 510

Arg Ser Lys Leu Ser Ser Leu Asn Lys Glu Val Ser Thr Leu Gln Asp
        515                 520                 525
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Thr Lys Val Met Thr Phe Lys Ala Ile Val Gln Leu Phe Val Leu Gly
530                 535                 540

Cys Ser Trp Gly Ile Gly Leu Phe Ile Phe Ile Glu Val Gly Lys Thr
545                 550                 555                 560

Val Arg Leu Ile Val Ala Tyr Leu Phe Thr Ile Ile Asn Val Leu Gln
                565                 570                 575

Gly Val Leu Ile Phe Met Val His Cys Leu Leu Asn Arg Gln Val Arg
            580                 585                 590

Met Glu Tyr Lys Lys Trp Phe His Arg Leu Arg Lys Glu Val Glu Ser
        595                 600                 605

Glu Ser Thr Glu Val Ser His Ser Thr Thr His Thr Lys Met Gly Leu
610                 615                 620

Ser Leu Asn Leu Glu Asn Phe Cys Pro Thr Gly Asn Leu His Asp Pro
625                 630                 635                 640

Ser Asp Ser Ile Leu Pro Ser Thr Glu Val Ala Gly Val Tyr Leu Ser
                645                 650                 655

Thr Pro Arg Ser His Met Gly Ala Glu Asp Val Asn Ser Gly Thr His
            660                 665                 670

Ala Tyr Trp Ser Arg Thr Ile Ser Asp
        675                 680
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<213> Homo sapiens

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His Cys Thr Cys Glu Asp Gly Phe Arg Ala Arg Ser Gly Arg Thr Tyr
            20                  25                  30

Phe His Asp Ser Ser Glu Lys Cys Glu Asp Ile Asn Glu Cys Glu Thr
        35                  40                  45

Gly Leu Ala Lys Cys Lys Tyr Lys Ala Tyr Cys Arg Asn Lys Val Gly
    50                  55                  60

Gly Tyr Ile Cys Ser Cys Leu Val Lys Tyr Thr Leu Phe Asn Phe Leu
65                  70                  75                  80

Ala Gly Ile Ile Asp Tyr Asp His Pro Asp Cys Tyr Glu Asn Asn Ser
                85                  90                  95

Gln Gly Thr Thr Gln Ser Asn Val Asp Ile Trp Glu Asn Leu Arg Arg
            100                 105                 110
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Asn Gly Ser Arg Glu Asp Phe Ala Arg Arg Ala Thr Gln Leu Ile Gln
        115                 120                 125

Ser Val Glu Leu Ser Ile Trp Asn Ala Ser Phe Ala Ser Pro Gly Lys
    130                 135                 140

Gly Gln Ile Ser Glu Phe Asp Ile Val Tyr Glu Thr Lys Arg Cys Asn
145                 150                 155                 160

Glu Thr Arg Glu Asn Ala Phe Leu Glu Ala Gly Asn Asn Thr Met Asp
                165                 170                 175

Ile Asn Cys Ala Asp Ala Leu Lys Gly Asn Leu Arg Glu Ser Thr Ala
            180                 185                 190

Val Ala Leu Ile Thr Tyr Gln Ser Leu Gly Asp Ile Leu Asn Ala Ser
        195                 200                 205

Phe Phe Ser Lys Arg Lys Gly Met Gln Glu Val Lys Leu Asn Ser Tyr
    210                 215                 220

Val Val Ser Gly Thr Val Gly Leu Lys Glu Lys Ile Ser Leu Ser Glu
225                 230                 235                 240

Pro Val Phe Leu Thr Phe Arg His Asn Gln Pro Gly Asp Lys Arg Thr
                245                 250                 255

Lys His Ile Cys Val Tyr Trp Glu Gly Ser Glu Gly Gly Arg Trp Ser
            260                 265                 270

Thr Glu Gly Cys Ser His Val His Ser Asn Gly Ser Tyr Thr Lys Cys
        275                 280                 285

Lys Cys Phe His Leu Ser Ser Phe Ala Val Leu Val Ala Leu Ala Pro
    290                 295                 300

Lys Glu Asp Pro Val Leu Thr Val Ile Thr Gln Val Gly Leu Thr Ile
305                 310                 315                 320

Ser Leu Leu Cys Leu Phe Leu Ala Ile Leu Thr Phe Leu Leu Cys Arg
                325                 330                 335

Pro Ile Gln Asn Thr Ser Thr Ser Leu His Leu Glu Leu Ser Leu Cys
            340                 345                 350

Leu Phe Leu Ala His Leu Leu Phe Leu Thr Gly Ile Asn Arg Thr Glu
        355                 360                 365

Pro Glu Val Leu Cys Ser Ile Ile Ala Gly Leu Leu His Phe Leu Tyr
    370                 375                 380

Leu Ala Cys Phe Thr Trp Met Leu Leu Glu Gly Leu His Leu Phe Leu
385                 390                 395                 400

Thr Val Arg Asn Leu Lys Val Ala Asn Tyr Thr Ser Thr Gly Arg Phe
                405                 410                 415
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Lys Lys Arg Phe Met Tyr Pro Val Gly Tyr Gly Ile Pro Ala Val Ile
420 425 430

Ile Ala Val Ser Ala Ile Val Gly Pro Gln Asn Tyr Gly Thr Phe Thr
435 440 445

His Cys Trp Leu Lys Leu Asp Lys Gly Phe Ile Trp Ser Phe Met Gly
450 455 460

Pro Val Ala Val Ile Ile Leu Ile Asn Leu Val Phe Tyr Phe Gln Val
465 470 475 480

Leu Trp Ile Leu Arg Ser Lys Leu Ser Ser Leu Asn Lys Glu Val Ser
485 490 495

Thr Ile Gln Asp Thr Arg Val Met Thr Phe Lys Ala Ile Ser Gln Leu
500 505 510

Phe Ile Leu Gly Cys Ser Trp Gly Leu Gly Phe Phe Met Val Glu Glu
515 520 525

Val Gly Lys Thr Ile Gly Ser Ile Ile Ala Tyr Ser Phe Thr Ile Ile
530 535 540

Asn Thr Leu Gln Gly Val Leu Leu Phe Val Val His Cys Leu Leu Asn
545 550 555 560

Arg Gln Val Arg Met Glu Tyr Lys Lys Trp Phe Ser Gly Met Arg Lys
565 570 575

Gly Val Glu Thr Glu Ser Thr Glu Met Ser Arg Ser Thr Thr Gln Thr
580 585 590

Lys Thr Glu Glu Val Gly Lys Ser Ser Glu Ile Phe His Lys Gly Gly
595 600 605

Thr Ala Ser Ser Ser Ala Glu Ser Thr Lys Gln Pro Gln Pro Gln Val
610 615 620

His Leu Val Ser Ala Ala Trp Leu Lys Met Asn
625 630 635

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<212> DNA
<213> Homo sapiens

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<212> PRT
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Asn Asn Cys Arg Asp Ser Thr Leu Cys Pro Ala Tyr Ala Thr Cys Thr
35 40 45

Asn Thr Val Asp Ser Tyr Tyr Cys Thr Cys Lys Gln Gly Phe Leu Ser
50 55 60

Ser Asn Gly Gln Asn His Phe Lys Asp Pro Gly Val Arg Cys Lys Asp
65 70 75 80

Ile Asp Glu Cys Ser Gln Ser Pro Gln Pro Cys Gly Pro Asn Ser Ser
85 90 95

Cys Lys Asn Leu Ser Gly Arg Tyr Lys Cys Ser Cys Leu Asp Gly Phe
100 105 110

Ser Ser Pro Thr Gly Asn Asp Trp Val Pro Gly Lys Pro Gly Asn Phe
115 120 125

Ser Cys Thr Asp Ile Asn Glu Cys Leu Thr Ser Arg Val Cys Pro Glu
130 135 140

His Ser Asp Cys Val Asn Ser Met Gly Ser Tyr Ser Cys Ser Cys Gln
145 150 155 160

Val Gly Phe Ile Ser Arg Asn Ser Thr Cys Glu Asp Val Asn Glu Cys
165 170 175

Ala Asp Pro Arg Ala Cys Pro Glu His Ala Thr Cys Asn Asn Thr Val
180 185 190

Gly Asn Tyr Ser Cys Phe Cys Asn Pro Gly Phe Glu Ser Ser Ser Gly
195 200 205

His Leu Ser Cys Gln Gly Leu Lys Ala Ser Cys Glu Asp Ile Asp Glu
210 215 220

Cys Thr Glu Met Cys Pro Ile Asn Ser Thr Cys Thr Asn Thr Pro Gly
225 230 235 240

Ser Tyr Phe Cys Thr Cys His Pro Gly Phe Ala Pro Ser Ser Gly Gln
245 250 255

Leu Asn Phe Thr Asp Gln Gly Val Glu Cys Arg Asp Ile Asp Glu Cys
260 265 270

Arg Gln Asp Pro Ser Thr Cys Gly Pro Asn Ser Ile Cys Thr Asn Ala
275 280 285

Leu Gly Ser Tyr Ser Cys Gly Cys Ile Val Gly Phe His Pro Asn Pro
290 295 300

Glu Gly Ser Gln Lys Asp Gly Asn Phe Ser Cys Gln Arg Val Leu Phe
305 310 315 320

Lys Cys Lys Glu Asp Val Ile Pro Asp Asn Lys Gln Ile Gln Gln Cys
325 330 335

Gln Glu Gly Thr Ala Val Lys Pro Ala Tyr Val Ser Phe Cys Ala Gln
340 345 350

Ile Asn Asn Ile Phe Ser Val Leu Asp Lys Val Cys Glu Asn Lys Thr
355 360 365

Thr Val Val Ser Leu Lys Asn Thr Thr Glu Ser Phe Val Pro Val Leu
370 375 380

Lys Gln Ile Ser Met Trp Thr Lys Phe Thr Lys Glu Glu Thr Ser Ser
385 390 395 400

Leu Ala Thr Val Phe Leu Glu Ser Val Glu Ser Met Thr Leu Ala Ser
405 410 415

Phe Trp Lys Pro Ser Ala Asn Val Thr Pro Ala Val Arg Ala Glu Tyr
420 425 430

Leu Asp Ile Glu Ser Lys Val Ile Asn Lys Glu Cys Ser Glu Glu Asn
435 440 445

Val Thr Leu Asp Leu Val Ala Lys Gly Asp Lys Met Lys Ile Gly Cys
450 455 460

Ser Thr Ile Glu Glu Ser Glu Ser Thr Glu Thr Thr Gly Val Ala Phe
465 470 475 480

Val Ser Phe Val Gly Met Glu Ser Val Leu Asn Glu Arg Phe Phe Gln
485 490 495

Asp His Gln Ala Pro Leu Thr Thr Ser Glu Ile Lys Leu Lys Met Asn
500 505 510

Ser Arg Val Val Gly Gly Ile Met Thr Gly Glu Lys Lys Asp Gly Phe
515 520 525

Ser Asp Pro Ile Ile Tyr Thr Leu Glu Asn Val Gln Pro Lys Gln Lys
530 535 540

Phe Glu Arg Pro Ile Cys Val Ser Trp Ser Thr Asp Val Lys Gly Gly
545 550 555 560

Arg Trp Thr Ser Phe Gly Cys Val Ile Leu Glu Ala Ser Glu Thr Tyr
565 570 575

Thr Ile Cys Ser Cys Asn Gln Met Ala Asn Leu Ala Val Ile Met Ala
580 585 590

Ser Gly Glu Leu Thr Met Asp Phe Ser Leu Tyr Ile Ile Ser His Val
595 600 605

Gly Ile Ile Ile Ser Leu Val Cys Leu Val Leu Ala Ile Ala Thr Phe
610 615 620

Leu Leu Cys Arg Ser Ile Arg Asn His Asn Thr Tyr Leu His Leu His
625 630 635 640

Leu Cys Val Cys Leu Leu Leu Ala Lys Thr Leu Phe Leu Ala Gly Ile
645 650 655

His Lys Thr Asp Asn Lys Thr Gly Cys Ala Ile Ile Ala Gly Phe Leu

660 665 670

His Tyr Leu Phe Leu Ala Cys Phe Phe Trp Met Leu Val Glu Ala Val
675 680 685

Ile Leu Phe Leu Met Val Arg Asn Leu Lys Val Val Asn Tyr Phe Ser
690 695 700

Ser Arg Asn Ile Lys Met Leu His Ile Cys Ala Phe Gly Tyr Gly Leu
705 710 715 720

Pro Met Leu Val Val Val Ile Ser Ala Ser Val Gln Pro Gln Gly Tyr
725 730 735

Gly Met His Asn Arg Cys Trp Leu Asn Thr Glu Thr Gly Phe Ile Trp
740 745 750

Ser Phe Leu Gly Pro Val Cys Thr Val Ile Val Ile Asn Ser Leu Leu
755 760 765

Leu Thr Trp Thr Leu Trp Ile Leu Arg Gln Arg Leu Ser Ser Val Asn
770 775 780

Ala Glu Val Ser Thr Leu Lys Asp Thr Arg Leu Leu Thr Phe Lys Ala
785 790 795 800

Phe Ala Gln Leu Phe Ile Leu Gly Cys Ser Trp Val Leu Gly Ile Phe
805 810 815

Gln Ile Gly Pro Val Ala Gly Val Met Ala Tyr Leu Phe Thr Ile Ile
820 825 830

Asn Ser Leu Gln Gly Ala Phe Ile Phe Leu Ile His Cys Leu Leu Asn
835 840 845

Gly Gln Val Arg Glu Glu Tyr Lys Arg Trp Ile Thr Gly Lys Thr Lys
850 855 860

Pro Ser Ser Gln Ser Gln Thr Ser Arg Ile Leu Leu Ser Ser Met Pro
865 870 875 880

Ser Ala Ser Lys Thr Gly
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Val Asn Glu Cys Gln Asp Thr Thr Thr Cys Pro Ala Tyr Ala Thr Cys

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     50                  55                  60

Ser Ser Asn Gly Gln Thr Asn Phe Gln Gly Pro Gly Val Glu Cys Gln
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Asp Val Asn Glu Cys Leu Gln Ser Asp Ser Pro Cys Gly Pro Asn Ser
                 85                  90                  95

Val Cys Thr Asn Ile Leu Gly Arg Ala Lys Cys Ser Cys Leu Arg Gly
            100                 105                 110

Phe Ser Ser Ser Thr Gly Lys Asp Trp Ile Leu Gly Ser Leu Asp Asn
        115                 120                 125

Phe Leu Cys Ala Asp Val Asp Glu Cys Leu Thr Ile Gly Ile Cys Pro
    130                 135                 140

Lys Tyr Ser Asn Cys Ser Asn Ser Val Gly Ser Tyr Ser Cys Thr Cys
145                 150                 155                 160

Gln Pro Gly Phe Val Leu Asn Gly Ser Ile Cys Glu Asp Glu Asp Glu
                165                 170                 175

Cys Val Thr Arg Asp Val Cys Pro Glu His Ala Thr Cys His Asn Thr
            180                 185                 190

Leu Gly Ser Tyr Tyr Cys Thr Cys Asn Ser Gly Leu Glu Ser Ser Gly
        195                 200                 205

Gly Gly Pro Met Phe Gln Gly Leu Asp Glu Ser Cys Glu Asp Val Asp
    210                 215                 220

Glu Cys Ser Arg Asn Ser Thr Leu Cys Gly Pro Thr Phe Ile Cys Ile
225                 230                 235                 240

Asn Thr Leu Gly Ser Tyr Ser Cys Ser Cys Pro Ala Gly Phe Ser Leu
                245                 250                 255

Pro Thr Phe Gln Ile Leu Gly His Pro Ala Asp Gly Asn Cys Thr Asp
            260                 265                 270

Ile Asp Glu Cys Asp Asp Thr Cys Pro Leu Asn Ser Ser Cys Thr Asn
        275                 280                 285

Thr Ile Gly Ser Tyr Phe Cys Thr Cys His Pro Gly Phe Ala Ser Ser
    290                 295                 300

Asn Gly Gln Leu Asn Phe Lys Asp Leu Glu Val Thr Cys Glu Asp Ile
305                 310                 315                 320

Asp Glu Cys Thr Gln Asp Pro Leu Gln Cys Gly Leu Asn Ser Val Cys
                325                 330                 335

Thr Asn Val Pro Gly Ser Tyr Ile Cys Gly Cys Leu Pro Asp Phe Gln
            340                 345                 350
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Gln Gln Cys Gln Ala Val Gln Gly Arg Asp Leu Gly Tyr Ala Ser Phe
385                 390                 395                 400

Cys Thr Leu Val Asn Ala Thr Phe Thr Ile Leu Asp Asn Thr Cys Glu
                405                 410                 415

Asn Lys Ser Ala Pro Val Ser Leu Gln Ser Ala Ala Thr Ser Val Ser
            420                 425                 430

Leu Val Leu Glu Gln Ala Thr Thr Trp Phe Glu Leu Ser Lys Glu Glu
        435                 440                 445

Thr Ser Thr Leu Gly Thr Ile Leu Leu Glu Thr Val Glu Ser Thr Met
    450                 455                 460

Leu Ala Ala Leu Leu Ile Pro Ser Gly Asn Ala Ser Gln Met Ile Gln
465                 470                 475                 480

Thr Glu Tyr Leu Asp Ile Glu Ser Lys Val Ile Asn Glu Glu Cys Lys
                485                 490                 495

Glu Asn Glu Ser Ile Asn Leu Ala Ala Arg Gly Asp Lys Met Asn Val
            500                 505                 510

Gly Cys Phe Ile Ile Lys Glu Ser Val Ser Thr Gly Ala Pro Gly Val
        515                 520                 525

Ala Phe Val Ser Phe Ala His Met Glu Ser Val Leu Asn Glu Arg Phe
    530                 535                 540

Phe Glu Asp Gly Gln Ser Phe Arg Lys Leu Arg Met Asn Ser Arg Val
545                 550                 555                 560

Val Gly Gly Thr Val Thr Gly Glu Lys Lys Glu Asp Phe Ser Lys Pro
                565                 570                 575

Ile Ile Tyr Thr Leu Gln His Ile Gln Pro Lys Gln Lys Ser Glu Arg
            580                 585                 590

Pro Ile Cys Val Ser Trp Asn Thr Asp Val Glu Asp Gly Arg Trp Thr
        595                 600                 605

Pro Ser Gly Cys Glu Ile Val Glu Ala Ser Glu Thr His Thr Val Cys
    610                 615                 620

Ser Cys Asn Arg Met Ala Asn Leu Ala Ile Ile Met Ala Ser Gly Glu
625                 630                 635                 640

Leu Thr Met Glu Phe Ser Leu Tyr Ile Ile Ser His Val Gly Thr Val
                645                 650                 655
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Ile Ser Leu Val Cys Leu Ala Leu Ala Ile Ala Thr Phe Leu Leu Cys
            660                 665                 670

Arg Ala Val Gln Asn His Asn Thr Tyr Met His Leu His Leu Cys Val
        675                 680                 685

Cys Leu Phe Leu Ala Lys Ile Leu Phe Leu Thr Gly Ile Asp Lys Thr
    690                 695                 700

Asp Asn Gln Thr Ala Cys Ala Ile Ile Ala Gly Phe Leu His Tyr Leu
705                 710                 715                 720

Phe Leu Ala Cys Phe Phe Trp Met Leu Val Glu Ala Val Met Leu Phe
                725                 730                 735

Leu Met Val Arg Asn Leu Lys Val Val Asn Tyr Phe Ser Ser Arg Asn
            740                 745                 750

Ile Lys Met Leu His Leu Cys Ala Phe Gly Tyr Gly Leu Pro Val Leu
        755                 760                 765

Val Val Ile Ile Ser Ala Ser Val Gln Pro Arg Gly Tyr Gly Met His
    770                 775                 780

Asn Arg Cys Trp Leu Asn Thr Glu Thr Gly Phe Ile Trp Ser Phe Leu
785                 790                 795                 800

Gly Pro Val Cys Met Ile Ile Thr Ile Asn Ser Val Leu Leu Ala Trp
                805                 810                 815

Thr Leu Trp Val Leu Arg Gln Lys Leu Cys Ser Val Ser Ser Glu Val
            820                 825                 830

Ser Lys Leu Lys Asp Thr Arg Leu Leu Thr Phe Lys Ala Ile Ala Gln
        835                 840                 845

Ile Phe Ile Leu Gly Cys Ser Trp Val Leu Gly Ile Phe Gln Ile Gly
    850                 855                 860

Pro Leu Ala Ser Ile Met Ala Tyr Leu Phe Thr Ile Ile Asn Ser Leu
865                 870                 875                 880

Gln Gly Ala Phe Ile Phe Leu Ile His Cys Leu Leu Asn Arg Gln Val
                885                 890                 895

Arg Asp Glu Tyr Lys Lys Leu Leu Thr Arg Lys Thr Asp Leu Ser Ser
            900                 905                 910

His Ser Gln Thr Ser Gly Ile Leu Leu Ser Ser Met Pro Ser Thr Ser
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Lys Met Gly
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<212> PRT

<213> Homo sapiens

<400> 7

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Gly Ala Glu Thr Gln Asp Ser Arg Gly Cys Ala Arg Val Cys Pro Gln
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Asn Ser Ser Cys Val Asn Ala Thr Ala Cys Arg Cys Asn Pro Gly Phe
        35                  40                  45

Ser Ser Phe Ser Glu Ile Ile Thr Thr Pro Thr Glu Thr Cys Asp Asp
    50                  55                  60

Ile Asn Glu Cys Ala Thr Pro Ser Lys Val Ser Cys Gly Lys Phe Ser
65                  70                  75                  80

Asp Cys Trp Asn Thr Glu Gly Ser Tyr Asp Cys Val Cys Ser Pro Gly
                85                  90                  95

Tyr Glu Pro Val Ser Gly Ala Lys Thr Phe Lys Asn Glu Ser Glu Asn
            100                 105                 110

Thr Cys Gln Asp Glu Cys Ser Ser Gly Gln His Gln Cys Asp Ser Ser
        115                 120                 125

Thr Val Cys Phe Asn Thr Val Gly Ser Tyr Ser Cys Arg Cys Arg Pro
    130                 135                 140

Gly Trp Lys Pro Arg His Gly Ile Pro Asn Asn Gln Lys Asp Thr Val
145                 150                 155                 160

Cys Glu Asp Met Thr Phe Ser Thr Trp Thr Pro Pro Pro Gly Val His
                165                 170                 175

Ser Gln Thr Leu Ser Arg Phe Phe Asp Lys Val Gln Asp Leu Gly Arg
            180                 185                 190

Asp Ser Lys Thr Ser Ser Ala Glu Val Thr Ile Gln Asn Val Ile Lys
        195                 200                 205

Leu Val Asp Glu Leu Met Glu Ala Pro Gly Asp Val Glu Ala Leu Ala
    210                 215                 220

Pro Pro Val Arg His Leu Ile Ala Thr Gln Leu Leu Ser Asn Leu Glu
225                 230                 235                 240

Asp Ile Met Arg Ile Leu Ala Lys Ser Leu Pro Lys Gly Pro Phe Thr
                245                 250                 255

Tyr Ile Ser Pro Ser Asn Thr Glu Leu Thr Leu Met Ile Gln Glu Arg
            260                 265                 270

Gly Asp Lys Asn Val Thr Met Gly Gln Ser Ser Ala Arg Met Lys Leu
        275                 280                 285

Asn Trp Ala Val Ala Ala Gly Ala Glu Asp Pro Gly Pro Ala Val Ala
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290 295 300

Gly Ile Leu Ser Ile Gln Asn Met Thr Thr Leu Leu Ala Asn Ala Ser
305 310 315 320

Leu Asn Leu His Ser Lys Lys Gln Ala Glu Leu Glu Glu Ile Tyr Glu
325 330 335

Ser Ser Ile Arg Gly Val Gln Leu Arg Arg Leu Ser Ala Val Asn Ser
340 345 350

Ile Phe Leu Ser His Asn Asn Thr Lys Glu Leu Asn Ser Pro Ile Leu
355 360 365

Phe Ala Phe Ser His Leu Glu Ser Ser Asp Gly Glu Ala Gly Arg Asp
370 375 380

Pro Pro Ala Lys Asp Val Met Pro Gly Pro Arg Gln Glu Leu Leu Cys
385 390 395 400

Ala Phe Trp Lys Ser Asp Ser Asp Arg Gly Gly His Trp Ala Thr Glu
405 410 415

Val Cys Gln Val Leu Gly Ser Lys Asn Gly Ser Thr Thr Cys Gln Cys
420 425 430

Ser His Leu Ser Ser Phe Thr Ile Leu Met Ala His Tyr Asp Val Glu
435 440 445

Asp Trp Lys Leu Thr Leu Ile Thr Arg Val Gly Leu Ala Leu Ser Leu
450 455 460

Phe Cys Leu Leu Leu Cys Ile Leu Thr Phe Leu Leu Val Arg Pro Ile
465 470 475 480

Gln Gly Ser Arg Thr Thr Ile His Leu His Leu Cys Ile Cys Leu Phe
485 490 495

Val Gly Ser Thr Ile Phe Leu Ala Gly Ile Glu Asn Glu Gly Gly Gln
500 505 510

Val Gly Leu Arg Cys Arg Leu Val Ala Gly Leu Leu His Tyr Cys Phe
515 520 525

Leu Ala Ala Phe Cys Trp Met Ser Leu Glu Gly Leu Glu Leu Tyr Phe
530 535 540

Leu Val Val Arg Val Phe Gln Gly Gln Gly Leu Ser Thr Arg Trp Leu
545 550 555 560

Cys Leu Ile Gly Tyr Gly Val Pro Leu Leu Ile Val Gly Val Ser Ala
565 570 575

Ala Ile Tyr Ser Lys Gly Tyr Gly Arg Pro Arg Tyr Cys Trp Leu Asp
580 585 590

Phe Glu Gln Gly Phe Leu Trp Ser Phe Leu Gly Pro Val Thr Phe Ile
595 600 605

Ile Leu Cys Asn Ala Val Ile Phe Val Thr Thr Val Trp Lys Leu Thr
610 615 620

Gln Lys Phe Ser Glu Ile Asn Pro Asp Met Lys Lys Leu Lys Lys Ala
625 630 635 640

Arg Ala Leu Thr Ile Thr Ala Ile Ala Gln Leu Phe Leu Leu Gly Cys
645 650 655

Thr Trp Val Phe Gly Leu Phe Ile Phe Asp Asp Arg Ser Leu Val Leu
660 665 670

Thr Tyr Val Phe Thr Ile Leu Asn Cys Leu Gln Gly Ala Phe Leu Tyr
675 680 685

Leu Leu His Cys Leu Leu Asn Lys Lys Val Arg Glu Glu Tyr Arg Lys
690 695 700

Trp Ala Cys Leu Val Ala Gly Gly Ser Lys Tyr Ser Glu Phe Thr Ser
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Thr Thr Ser Gly Thr Gly His Asn Gln Thr Arg Ala Leu Arg Ala Ser
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Glu Ser Gly Ile
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<220>
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<210> 14
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<210> 15
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<213> Artificial Sequence

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<400> 17
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tgtctcattg cacctcttgg tttcat 26

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ccacaacagc acccactgt 19

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<211> 33
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<210> 21

<211> 33
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<210> 23
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<210> 24
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<210> 25
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<220>
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU00/01083

**A. CLASSIFICATION OF SUBJECT MATTER**

Int. Cl. [7]: C07K 14/435, 14/47, C07H 21/04, A61K 39/395, A61P 37/06, C12N 5/16, 5/22, C12Q 1/24, G01N 33/54

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)
IPC 7: As Above

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
ANGIS

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | The Journal of Biological Chemistry, Vol. 271, No. 1, issue of 5 January 1996 (U.S.A.), Andrew J. McKnight et al., "Molecular Cloning of F4/80, A Murine Macrophage-restricted Cell Surface Glycoprotein with Homology to th G-protein-linked Transmembrane 7 Hormone Receptor Family", pages 486 to 489<br>See peptide in Fig. 1. Matching for SEQ. ID. No.1: positives 70% and identities 53%, and SEQ. ID. No.2: positives 73% and 55% identities. | 1 - 4, 7 -11 |

[X] Further documents are listed in the continuation of Box C [ ] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing |
|---|---|
| 19 October 2000 | 01 NOV 2000 |

| Name and mailing address of the ISA/AU | Authorized officer |
|---|---|
| AUSTRALIAN PATENT OFFICE<br>PO BOX 200, WODEN ACT 2606, AUSTRALIA<br>E-mail address: pct@ipaustralia.gov.au<br>Facsimile No. (02) 6285 3929 | GAVIN THOMPSON<br>Telephone No : (02) 6283 2240 |

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU00/01083

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| | Genomics, Vol. 67, No. 2, accepted 25 April 2000, (San Diego, U.S.A.), His-Hsien Lin et al., "Human EMR2, a Novel EGF-TM7 Molecule on Chromosome19p13.1 is closely related to CD97", pages 188 to 200 | |
| P, X | See figure on page 191. Matching for SEQ. ID. No. 1: positives 79% and identities 63%, and SEQ. ID. No. 2: positives 80% and identities 65%. | 1 - 4, 7 -11 |
| | Genomics, Vol. 26, 1995, Veronique Baud et al, "EMR1, an Unusual Member in the Family of Hormone Receptors with Seven Transmembrane Segments", pages 334 to 344 | |
| X | See Fig. 1. Matching for SEQ. ID. No. 1: positives 70% and identities 54%, and SEQ. ID. No. 2: positives 72% and identities 55%. | 1 - 4, 7 - 11 |